(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
21 April 2005 (21.04.2005)

PCT

(10) International Publication Number
WO 2005/034995 A1

(51) International Patent Classification[7]: A61K 39/395, G01N 21/00, 33/53

(21) International Application Number: PCT/US2003/031057

(22) International Filing Date: 30 September 2003 (30.09.2003)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
60/502,032 10 September 2003 (10.09.2003) US

(71) Applicant *(for all designated States except US)*: **CHIRON CORPORATION** [US/US]; 4560 Horton Street, Emeryville, CA 94608 (US).

(72) Inventors; and

(75) Inventors/Applicants *(for US only)*: **ABRIGNANI, Sergio** [IT/IT]; c/o Chiron Corporation, P.O. 8097, Emeryville, CA 94662-8097 (US). **COHEN, Fred** [US/US]; c/o Chiron Corporation, P.O. Box 8097, Emeryville, CA 94662-8097 (US). **MICHELITSCH, Melissa, D.** [US/US]; c/o Chiron Corporation, P.O. Box 8097, Emeryville, CA 94662-8097 (US). **HU, Celine, Yuan-Hwei** [—/US]; c/o Chiron Corporation, P.O. Box 8097, Emeryville, CA 94662-8097 (US). **PHELPS, Bruce** [US/US]; c/o Chiron Corporation, P.O. Box 8097, Emeryville, CA 94662-8097 (US).

(74) Agents: **LILLIS, Marcella** et al.; Chiron Corporation, Intellectual Property, P.O. Box 8097, Emeryville, CA 94662-8097 (US).

(81) Designated States *(national)*: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NI, NO, NZ, OM, PG, PH, PL, PT, RO, RU, SC, SD, SE, SG, SK, SL, SY, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, YU, ZA, ZM, ZW.

(84) Designated States *(regional)*: ARIPO patent (GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZM, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HU, IE, IT, LU, MC, NL, PT, RO, SE, SI, SK, TR), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).

Published:
— *with international search report*

For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

(54) Title: PRION CHIMERAS AND USES THEREOF

(57) Abstract: The invention relates to prions chimeras, antibodies specific to prions, polynucleotides encoding these chimeras and antibodies, and to methods of generating antibodies using chimeras. The invention further relates to methods of using such chimeras and antibodies to detect the presence of pathogenic prions in a biological sample and to methods of using such antibodies, chimeras and/or polynucleotides as components of a therapeutic or prophylactic vaccine.

WO 2005/034995 A1

PRION CHIMERAS AND USES THEREOF

FIELD OF THE INVENTION

The invention relates to prion chimeras, polynucleotides encoding these chimeras, methods of generating antibodies using such chimeras and polynucleotides, and to antibodies generated using these methods. The invention further relates to methods of using such chimeras, polynucleotides and/or antibodies to detect the presence of pathogenic prions in a biological sample and to methods of using such antibodies, polynucleotides and/or prion protein chimeras to raise an immune response and/or as components in a therapeutic or prophylactic vaccine.

BACKGROUND

Conformational diseases include a variety of unrelated diseases, including transmissible spongiform encephalopathies (*e.g.,* prion diseases) and Alzheimer disease, arising from aberrant conformational transition of a protein which in turn leads to self-association of the aberrant protein forms, with consequent tissue deposition and damage. These diseases also share striking similarities in clinical presentations, typically a slow and insidious onset when the transition is

In humans, prion diseases include Creutzfeldt-Jakob disease (CJD), Gerstmann-Straussler-Scheinker syndrome, Fatal Familial Insomnia, and Kuru (see, e.g., Harrison's Principles of Internal Medicine, Isselbacher et al., eds., McGraw-Hill, Inc. New York, (1994); Medori et al. 1992 N. Engl. J. Med., 326: 444-9.). In animals the transmissible spongiform encephalopathies (TSEs) include sheep scrapie, bovine spongiform encephalopathy (BSE), transmissible mink encephalopathy, and chronic wasting disease of captive mule deer and elk (Gajdusek, (1990) Subacute Spongiform Encephalopathies: Transmissible Cerebral Amyloidoses Caused by Unconventional Viruses. Pp. 2289-2324 In: Virology, Fields, ed. New York: Raven Press, Ltd.). Transmissible spongiform encephalopathies are characterized by the same hallmarks: a spongiform degeneration, reactive gliosis in the cortical and subcortical gray matters of the brain, and transmission when experimentally inoculated into laboratory animals including primates, rodents, and transgenic mice.

The term "prion" was originally coined as a reference to a **pro**teinaceous **in**fectious agent that lacks nucleic acid. (See, *e.g.,* Bolton, McKinley et al. (1982) Science 218:1309-1311; Prusiner, Bolton et al. (1982) Biochemistry 21:6942-6950; McKinley, Bolton et al. (1983) Cell 35:57-62 and Prusiner, "Prions", *Proc. Natl. Acad. Sci. USA*, 95: 13363-13383 (1998) (hereinafter referred to as "Prusiner, "Prions""")). Complete prion protein-encoding genes have since been cloned, sequenced and expressed in transgenic animals. PrP^C is encoded by a single-copy host gene. See, *e.g.,* Basler, Oesch et al. (1986) Cell 46:417-428. At least 20 different mutations in the human PrP gene have been found to be associated with inherited prion diseases. It is thought likely that these inherited forms of PrP^{Sc} are also pathogenic should they be transmitted to another host.

The key characteristic of conformational diseases is the presence of an abnormally shaped protein. In the case of prion disease, the normal form of prion protein (PrP^C) becomes the abnormally shaped protein (PrP^{SC}). See, *e.g.,* Cohen & Pruisner (1998) *Ann Rev. Biochem.* 67:793-819; Pan et al. (1993) Proc Natl Acad Sci USA 90:10962-10966; Safar et al. (1993) J Biol Chem 268:20276-20284. Optical spectroscopy and crystallography studies have recently revealed that disease-related forms of prions (pathogenic prions) are substantially enriched in beta-sheet structure as compared to the predominantly alpha-helical folded non-disease forms. See, *e.g.,* Wille et al. (2001) *Proc. Nat'l Acad. Sci. USA* 99:3563-3568. In particular, it is believed that PrP^C generally contains about 40% α-helix and little β-sheet, while PrP^{SC} contains about 30% α-helix and about 45% β-sheet. The proposed PrP^{SC} parallel β-helix structure is thought to be an unusually stable conformation, similar to β-helical structures generally found in proteins subjected to harsh, denaturing environments such as bacterial or viral virulence factors or plant pollens. In addition, it is now suggested that the proposed β-helical fold may be formed in a two-state manner analogous to α-helix formation. Based on this predicted structure, it has been suggested that the conversion of PrP^C to PrP^{SC} may result in the stabilization of a proto-β-helical motif by a neighboring PrP^{SC} molecule and subsequent extension to form the complete β-helical structure. The structural changes appear to be followed by alterations in the biochemical properties: PrP^C is soluble in non-denaturing detergents, PrP^{SC} is insoluble; PrP^C is readily digested by proteases, while PrP^{SC} is partially resistant, resulting in the formation of an N-terminally truncated fragment known as "PrPres" (Baldwin et al. (1995); Cohen & Pruisner (1995)), "PrP 27-30" (27-30 kDa) or "PK-resistant" (proteinase K resistant) form.

Prion-related diseases are thought to be initiated by a variety of mechanisms, including infection from another mammalian host, genetic mutations in the PrP gene, and

spontaneous conformational changes. It is hypothesized that PrP^{SC} acts as a template for the conversion of PrP^{C} to PrP^{SC}. Only a minute amount of PrP^{SC} is believed to be needed to start this process and eventually lead to irreversible neurodegenerative damage. Pathogenic prions have also been transmitted through improperly sterilized depth electrodes, transplanted corneas, human growth hormone (HGH) and gonadotrophin derived from cadaveric pituitaries, and dura matter grafts. In addition, transmission of pathogenic prions is also blamed in the BSE epidemic in Great Britain, where cattle were purportedly infected with prions through a meat and bone meal (MBM) fed primarily to dairy cows.

The tragic consequences of accidental transmission of these diseases (see, e.g., Gajdusek, Infectious Amyloids, and Prusiner Prions In Fields Virology. Fields, et al., eds. Lippincott-Ravin, Pub. Philadelphia (1996); Brown et al. (1992) Lancet, 340: 24-27), decontamination difficulties (Asher et al. (1986) pages 59-71 In: Laboratory Safety: Principles and Practices, Miller ed. Am. Soc. Microb.), and recent concern about bovine spongiform encephalopathy (British Med. J. 1995; 311: 1415-1421) underlie the urgency of having diagnostic tests that would identify humans and animals with transmissible spongiform encephalopathies as well as potential treatments for these diseases.

Definitively diagnosing these transmissible and amyloid-containing conditions before death of the subject has proven difficult. Histopathological examination of brain biopsies is risky to the subject and lesions and amyloid deposits can be missed depending on where the biopsy sample is taken from. PrP^{SC} is resistant to many proteases, and it is generally thought that this characteristic prevents pathogenic prion conformations from being processed and presented to antigen presenting cells (APC). In addition, the amino acid sequence similarity between non-pathogenic prions and pathogenic prions makes it difficult for a host's immune system to generate antibodies specific to PrP^{SC}. Indeed, prion-related diseases typically kill the host without any sign of an immune response to the pathogenic prion. U.S. Patent No. 6,562,341 is directed to a device including a solid support and an antibody that binds to native PrP^{SC} *in situ*.

Thus, there remains a need for compositions and methods for diagnosing and/or treating prion diseases.

SUMMARY

The present invention is related to prion chimeras that can be used to raise antibodies specific to PrP^{SC}. The antibodies and chimeras of the invention can be used in a wide range of applications, including as diagnostic tools to detect the presence of pathogenic prions in a

biological sample, in methods of generating immune response, and/or as components of a therapeutic or prophylactic vaccine.

In one aspect, the invention comprises a prion chimera comprising a prion polypeptide and a non-prion polypeptide. In certain embodiments, the prion polypeptide comprises an amino acid sequence of a prion protein that can adapt the β-helical conformation of pathogenic prion, for example, the sequences depicted in FIG. 1 (SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:6, SEQ ID NO:12, SEQ ID NO:7 or SEQ ID NO:13). Similarly, the non-prion polypeptide may be derived from a beta-helical protein. The beta-helical protein may be left- or right- handed, for example, pertactin or GCA (or fragments thereof), as depicted in FIGs. 2 and 7 (SEQ ID NO:3, SEQ ID NO:183, SEQ ID NO:8, SEQ ID NO:9, SEQ ID NO:4, SEQ ID NO:184, SEQ ID NO:10 and SEQ ID NO:11)). In certain embodiments, the prion chimeras are those depicted in FIGs. 3 through 6, 8 through 23 and 25 through 28 (SEQ ID NO:14-153 and SEQ ID NO:185-208). In other embodiments, the non-prion polypeptide of the chimera does not contain one or more of the amino acids of the non-prion protein that adapt the β-helix conformation. The prion chimeras are typically not, themselves, pathogenic and/or infectious. In other embodiments, a polynucleotide (*e.g.*, a DNA construct) encodes a prion chimera, for example the constructs shown in FIGs. 20-23 (SEQ ID NO:155-182).

In any of the chimeras described herein, the non-prion protein segment preferably allows the prion protein segment to adapt its β-helical structure in the prion chimera. Further, in any of the chimeras described herein, the component amino acid sequences (*e.g.*, non-prion sequences and prion sequences) may be co-linear (*e.g.*, the prion sequences are N- and/or C-terminal to the non-prion sequences). Alternatively, one component may be inserted into the other component. Further, the prion chimera may further comprise a tag sequence, for example, a histidine tag sequence as depicted in SEQ ID NO. 5 or SEQ ID NO:6.

In another aspect, the invention includes a method of generating antibodies specific to a pathogenic prion, the method comprising the step of administering to an animal a prion chimera, wherein the chimera comprises a prion protein or a fragment or derivative thereof; and a non-prion, β-helical protein or a fragment or derivative thereof. The method may also include the step of isolating the antibodies from the animal. In certain embodiments, the prion protein or fragment or derivative thereof has a β-helical conformation of a pathogenic prion. Any of the chimeras described herein may include prion proteins containing the amino acid sequences set forth in FIG 1 (SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:6, SEQ ID NO:12, SEQ ID NO:7 or SEQ ID NO:13). Further, in any of the chimeras described herein,

one or more amino acids of the β-helix of the β-helical protein may be replaced with the prion protein or fragment or derivative thereof. Any of the chimeras described herein may include non-prion proteins (*e.g.,* left handed or right handed β-helical proteins such as pertactin (P69) or γ carbonic anhydrase), for example, containing the amino acid sequences set forth in FIGs. 2 and 7 (SEQ ID NO:3, SEQ ID NO:183, SEQ ID NO:8, SEQ ID NO:9, SEQ ID NO:4, SEQ ID NO:184, SEQ ID NO:10 and SEQ ID NO:11). The prion chimera may further comprise a tag sequence, for example, a histidine tag sequence as depicted in SEQ ID NO. 5 or SEQ ID NO:6. In any of the methods described herein, specific antibodies may be from the animal. Prion chimeras include, for example, the chimeras depicted in Figures 3-6, 8-23 and 25-28 (SEQ ID NO:14-153 and 185-208). Any of the prion chimeras used to generate antibodies may also be encoded by polynucleotides, for example polynucleotides operably linked to control elements that are expressed to produce the prion chimera. The polynucleotide constructs encoding the prion chimeras may also include a signal sequence, for example a leader sequence derived from tpa. Exemplary polynucleotide constructs are depicted in Figures 20-23 (SEQ ID NO:155-182).

In another aspect, the invention includes antibodies specific a prion chimera, for example an antibody that is specific for the β-helical region of a pathogenic prion. In certain embodiments, the antibodies are generated by any of the methods described herein, for example, by administering one or more of the prion chimeras (or polynucleotides encoding these prion chimeras) to an animal (*e.g.,* non-human or human mammal) subject, by phage display techniques, or the like. The antibodies of the present invention may be monoclonal and polyclonal antibodies. Any of the antibodies described herein may also be encoded by one or more polynucleotides.

In yet another aspect, the invention includes a method for detecting the presence of a pathogenic prion in a biological sample, comprising (a) exposing the biological sample to any of the prion chimera-specific antibodies described herein (*e.g.,* an antibody generated by the methods described herein); and (b) detecting the presence or absence of the antibody bound to a pathogenic prion. Preferably, the antibody is directed against a β-helical region of a pathogenic prion.

In a still further aspect, the invention includes a method for detecting the presence of a pathogenic prion in a biological sample comprising: (a) exposing the biological sample suspected of containing a pathogenic prion to a prion chimera; and (b) detecting the presence or absence of the prion chimera bound to the pathogenic prion, if any, in the sample.

In another aspect, the invention includes a method for detecting a pathogenic prion in a biological sample, comprising: (a) providing a solid support comprising a first antibody (or prion chimera) bound thereto, wherein the first antibody specific to a prion chimera (*e.g.*, an antibody generated by the methods described herein); (b) exposing the solid support to a biological sample under conditions which allow pathogenic prions, when present in the biological sample, to bind to the first antibody or prion chimera; (c) exposing the solid support to a detectably labeled second antibody specific to pathogenic prions or detectably labeled prion chimera under conditions which allow the second antibody or detectably labeled prion chimera to bind to pathogenic prions bound by the first antibody or prion chimera; and (d) detecting complexes formed between the first antibody or prion chimera, a pathogenic prion from the biological sample and the second antibody or detectably labeled prion chimera, thereby detecting the presence of the pathogenic prion in the biological sample.

In yet another aspect, the invention includes a method for detecting the presence of a pathogenic prion in a biological sample comprising: (a) providing a solid support comprising a first antibody or prion chimera bound thereto, wherein the first antibody or prion chimera recognizes pathogenic and non-pathogenic prions; (b) exposing the solid support to a biological sample under conditions which allow prion proteins, when present in the biological sample, to bind to the first antibody or prion chimera; (c) exposing the solid support to a detectably labeled second antibody specific to a prion chimera (*e.g.*, labeled antibody generated according to the methods described herein) or a detectably labeled prion chimera that binds to pathogenic prions; and (d) detecting complexes formed between the first antibody, a pathogenic prion from the biological sample, and the second antibody or the detectably labeled prion chimera.

In another aspect, the invention includes a method for detecting the presence of a pathogenic prion in a biological sample comprising: (a) providing a solid support comprising a first antibody specific for a prion chimera (*e.g.*, an antibody generated according to the methods described herein) or prion chimera bound thereto, wherein the first antibody or prion chimera is specific to pathogenic prions; (b) combining the solid support with a detectably labeled first ligand or antigen, wherein the first antibody's or prion chimera's binding affinity to the detectably labeled first ligand or antigen is weaker than the first antibody's (or prion chimera's) binding affinity to a pathogenic prion; (c) combining a biological sample with the solid support under conditions which allow a pathogenic prion, when present in the biological sample, to bind to the first antibody (or prion chimera's) and replace the first ligand or

antigen; and (d) detecting complexes formed between the first antibody (or prion chimera) and the pathogenic prion from the biological sample.

In any of the detection methods described herein, the biological sample can be organs, whole blood, blood fractions, plasma, cerebrospinal fluid (CSF), urine, tears, tissue, organs, and/or biopsies. In certain embodiments, the biological sample is blood. In any of the detection methods described, the solid support can be, for example, nitrocellulose, polystyrene latex, polyvinyl fluoride, diazotized paper, nylon membranes, activated beads, and/or magnetically responsive beads.

In a still further aspect, the invention includes a solid support comprising at least one prion chimera or at least one antibody specific to pathogenic and/or non-pathogenic prions bound thereto, for example, an antibody generated according to the methods described herein that is specific for pathogenic prions (*e.g.*, directed against a β-helical region of a pathogenic prion). In certain embodiments, the solid supports as described herein are used in an immunoassay (*e.g.*, where the antibody bound the solid support is specific to pathogenic prions or where the antibody bound to the solid s support is specific to pathogenic and nonpathogenic prions). The solid support can comprise, for example, nitrocellulose, polystyrene latex, polyvinyl fluoride, diazotized paper, nylon membranes, activated beads, and magnetically responsive beads.

In another aspect, the invention includes a kit for detecting the presence of a pathogenic prion in a biological sample comprising: (a) a solid support comprising at least one antibody (*e.g.*, an antibody generated according to the methods described herein) or prion chimera specific to pathogenic prions bound thereto; and (b) ancillary reagents and, optionally, positive and negative controls.

In yet another aspect, the invention includes an immunogenic composition comprising a prion chimera and, optionally, an adjuvant, wherein the prion chimera comprises a prion protein or a fragment or derivative thereof and a non-prion β-helical protein or a fragment or derivative thereof. The prion protein may comprise, for example, a prion protein (or fragment thereof) that can adopt a β-helical conformation of a pathogenic prion protein (*e.g.*, the amino acid sequences set forth in SEQ ID NO:1; SEQ ID NO:2; SEQ ID NO:6, SEQ ID NO:12, SEQ ID NO:7 and/or SEQ ID NO:13). In any of the immunogenic compositions described herein, one or more amino acids of the β-helix of the β-helical protein are replaced with the prion protein or fragment or derivative thereof. Further, in any of the immunogenic compositions described herein the non-prion β-helical protein can be a left handed helical protein or a right handed helical protein or a fragment thereof, for example, pertactin (*e.g.*,

P69 pertactin) or γ carbonic anhydrase or fragments thereof (*e.g.*, SEQ ID NO:3; SEQ ID NO:4; SEQ ID NO:183; SEQ ID NO:8; SEQ ID NO:9; SEQ ID NO:10; SEQ ID NO:240 and/or SEQ ID NO:11). In certain embodiments, the immunogenic composition comprises one or more prion chimeras depicted in FIGs. 3-6, 8-23 and 25-28 (SEQ ID NO:14-153 and SEQ ID NO:185-208). In other embodiments, the immunogenic composition comprises one or more polynucleotides encoding prion chimeras depicted in FIGs. 20-23 (SEQ ID NO:155-182). Further, the adjuvant component may be encoded by a polynucleotide. The immunogenic compositions may also comprise combinations and/or mixtures of polypeptides and polynucleotides.

In another aspect, the invention includes a method of raising an immune response to an infectious prion comprising administering to an animal an immunologically effective amount of any of the immunogenic compositions described herein (*e.g.*, prion protein chimeras and/or polynucleotides encoding prion chimeras). Antibodies (or polynucleotide encoding these antibodies) as described herein may also be administered to a subject. In certain embodiments, one or more components of the immunogenic composition is encoded by a polynucleotide that is incorporated into a gene delivery vehicle, for example a viral vector (*e.g.*, alphaviral vector), a non-viral vector, a particular carrier (*e.g.*, PLG), and/or a liposome preparation.

In a still further aspect, the invention includes "prime-boost" type methods of inducing an immune response in a subject, for example methods comprising (a) administering a first composition comprising an immunogenic composition comprising a polynucleotide (*e.g.*, a polynucleotide encoding a prion chimera or a polynucleotide an antibody specific for pathogenic prions) in a priming step and (b) administering a second composition comprising a polypeptide (*e.g.*, a prion chimera or an antibody specific for pathogenic prions), as a booster, in an amount sufficient to induce an immune response in the subject.

In any of the methods of generating an immune response as described herein, one or more adjuvants may be included. Further, the subject may be a mammal, for example, a human. In any of these methods, one or more components may be administered intramuscularly, intramucosally, intranasally, subcutaneously, intradermally, transdermally, intravaginally, intrarectally, orally and/or intravenously. In any of the methods described herein, the immune response may be prophylactic and/or therapeutic.

These and other embodiments of the subject invention will readily occur to those of skill in the art in light of the disclosure herein.

BRIEF DESCRIPTION OF THE DRAWINGS

FIGs. 1A and 1B depict amino acid sequences of human and mouse PrPs. FIG 1A depicts a full-length human sequence (SEQ ID NO:1) and fragments extending from amino acid residues 135-155 (SEQ ID NO:6) and amino acid residues 126-154 (SEQ ID NO:12). FIG 1B depicts full-length mouse sequence (SEQ ID NO:2) and fragments extending from amino acid residues 135-155 (SEQ ID NO:7) and amino acid residues 126-154 (SEQ ID NO:13).

FIGs. 2A – 2B depict amino acid sequences of an exemplary non-prion sequence designated "P69" pertactin and derived from *Pertussis toxin*. FIG 2A depicts a full-length amino acid sequence of P69 (SEQ ID NO:3). FIG. 2B depicts fragments of SEQ ID NO:3 designated "P69 Control A" (SEQ ID NO:8) and "P69 Control B" (SEQ ID NO:9).

FIGs. 3A – 3D depict exemplary prion chimeras including "P69 control A" non-prion amino acid sequences and human PrP prion amino acid sequences (135-155). FIG. 3A depicts sequences of prion chimeras designated P69A/human Chimera No. 1 (SEQ ID NO:14) and P69A/human Chimera No. 2 (SEQ ID NO:15). FIG. 3B depicts sequences of prion chimeras designated P69A/human Chimera No. 3 (SEQ ID NO:16) and P69A/human Chimera No. 4 (SEQ ID NO:17). FIG. 3C depicts sequences of prion chimeras designated P69A/human Chimera No. 5 (SEQ ID NO:18) and P69A/human Chimera No. 6 (SEQ ID NO:19). FIG. 3D depicts sequences of prion chimeras designated P69A/human Chimera No. 7 (SEQ ID NO:20) and P69A/human Chimera No. 8 (SEQ ID NO:21). Chimeras designated P69A/human (135-155) Chimera No. 1-8 include sequences derived from P69 Control A (SEQ ID NO:8); sequences derived from human PrP (135-155) (SEQ ID NO:6); and a His tag.

FIGs. 4A – 4D depict exemplary prion chimeras including "P69 Control A" non-prion amino acid sequences and mouse PrP prion amino acid sequences (135-155). FIG. 4A depicts sequences of prion chimeras designated P69A/mouse Chimera No. 1 (SEQ ID NO:22) and P69A/mouse Chimera No. 2 (SEQ ID NO:23). FIG. 4B depicts sequences of prion chimeras designated P69A/mouse Chimera No. 3 (SEQ ID NO:24) and P69A/mouse Chimera No. 4 (SEQ ID NO:25). FIG. 4C depicts sequences of prion chimeras designated P69A/mouse Chimera No. 5 (SEQ ID NO:26) and P69A/mouse Chimera No. 6 (SEQ ID NO:27). FIG. 4D depicts sequences of prion chimeras designated P69A/mouse Chimera No. 7 (SEQ ID NO:28) and P69A/mouse Chimera No. 8 (SEQ ID NO:29). Chimeras designated P69A/mouse (135-155) Chimera No. 1-8 include sequences derived from P69 Control A

(SEQ ID NO:8); sequences derived from mouse PrP (135-155) (SEQ ID NO:7); and a His tag.

FIGs. 5A – 5D depict exemplary prion chimeras including "P69 control B" non-prion amino acid sequences and human PrP prion amino acid sequences (135-155). FIG. 5A depicts sequences of prion chimeras designated P69B/human Chimera No. 1 (SEQ ID NO:30) and P69B/human Chimera No. 2 (SEQ ID NO:31). FIG. 5B depicts sequences of prion chimeras designated P69B/human Chimera No. 3 (SEQ ID NO:32) and P69B/human Chimera No. 4 (SEQ ID NO:33). FIG. 5C depicts sequences of prion chimeras designated P69B/human Chimera No. 5 (SEQ ID NO:34) and P69B/human Chimera No. 6 (SEQ ID NO:35). FIG. 5D depicts sequences of prion chimeras designated P69B/human Chimera No. 7 (SEQ ID NO:36) and P69B/human Chimera No. 8 (SEQ ID NO:37). Chimeras designated P69B/human (135-155) Chimera No. 1-8 include sequences derived from P69 Control B (SEQ ID NO:9); sequences derived from human PrP (135-155) (SEQ ID NO:6); and a His tag.

FIGs. 6A – 6D depict exemplary prion chimeras including "P69 Control B" non-prion amino acid sequences and mouse PrP prion amino acid sequences (135-155). FIG. 6A depicts sequences of prion chimeras designated P69B/mouse Chimera No. 1 (SEQ ID NO:38) and P69B/mouse Chimera No. 2 (SEQ ID NO:39). FIG. 6B depicts sequences of prion chimeras designated P69B/mouse Chimera No. 3 (SEQ ID NO:40) and P69B/mouse Chimera No. 4 (SEQ ID NO:41). FIG. 6C depicts sequences of prion chimeras designated P69B/mouse Chimera No. 5 (SEQ ID NO:42) and P69B/mouse Chimera No. 6 (SEQ ID NO:43). FIG. 6D depicts sequences of prion chimeras designated P69B/mouse Chimera No. 7 (SEQ ID NO:44) and P69B/mouse Chimera No. 8 (SEQ ID NO:45). Chimeras designated P69B/mouse (135-155) Chimera No. 1-8 include sequences derived from P69 Control B (SEQ ID NO:9); sequences derived from mouse PrP (135-155) (SEQ ID NO:7); and a His tag.

FIG. 7 depicts an exemplary non-prion amino acid sequences derived from left-handed non-prion β-helical protein *Methanosarcina thermophila* γ carbonic anhydrase ("GCA"). SEQ ID NO:4 depicts the full length sequence of GCA; SEQ ID NO:183 depicts full length GCA with the leader sequence removed; SEQ ID NO:10 depicts a fragment of GCA designated "GCA Control A (GCAA);" SEQ ID NO:11 depicts a fragment of GCA designated as "GCA Control B (GCAB); and SEQ ID NO:184 depicts a fragment of GCA with the leader sequence removed designated "GCABnoL."

FIGs. 8A – 8B depict exemplary prion chimeras including "GCA Contol A" non-prion sequences and human PrP 135-155 sequences. FIG. 8A depicts sequences of prion chimeras designated GCAA/human Chimera No. 1 (SEQ ID NO:46); GCAA/human Chimera No. 2 (SEQ ID NO:47); and GCAA/human Chimera No. 3 (SEQ ID NO:48). FIG. 8B depicts sequences of prion chimeras designated GCAA/human Chimera No. 4 (SEQ ID NO:49); GCAA/human Chimera No. 5 (SEQ ID NO:50); and GCAA/human Chimera No. 6 (SEQ ID NO:51). Chimeras designated GCAA/human Chimera No. 1-6 include sequences derived from GCAA (SEQ ID NO:10); sequences derived from human PrP (135-155) (SEQ ID NO:6); and a His tag.

FIGs. 9A – 9B depict exemplary prion chimeras including "GCA Contol A" non-prion sequences and mouse PrP 135-155 sequences. FIG. 9A depicts sequences of prion chimeras designated GCAA/mouse Chimera No. 1 (SEQ ID NO:52); GCAA/mouse Chimera No. 2 (SEQ ID NO:53); and GCAA/mouse Chimera No. 3 (SEQ ID NO:54). FIG. 9B depicts sequences of prion chimeras designated GCAA/mouse Chimera No. 4 (SEQ ID NO:55); GCAA/mouse Chimera No. 5 (SEQ ID NO:56); and GCAA/mouse Chimera No. 6 (SEQ ID NO:57). Chimeras designated GCAA/mouse Chimera No. 1-6 include sequences derived from GCAA (SEQ ID NO:10); sequences derived from mouse PrP (135-155) (SEQ ID NO:7); and a His tag.

FIGs. 10A – 10B depict exemplary prion chimeras including "GCA Control B" non-prion sequences and human PrP 135-155 sequences. FIG. 10A depicts sequences of prion chimeras designated GCAB/human Chimera No. 1 (SEQ ID NO:58); GCAB/human Chimera No. 2 (SEQ ID NO:59); and GCAB/human Chimera No. 3 (SEQ ID NO:60). FIG. 10B depicts sequences of prion chimeras designated GCAB/human Chimera No. 4 (SEQ ID NO:61); GCAB/human Chimera No. 5 (SEQ ID NO:62); and GCAB/human Chimera No. 6 (SEQ ID NO:63). Chimeras designated GCAB/human (135-155) Chimera No. 1-6 include sequences derived from GCAB (SEQ ID NO:11); sequences derived from human PrP (135-155) (SEQ ID NO:6); and a His tag.

FIGs. 11A – 11B depict exemplary prion chimeras including "GCA Control B" non-prion sequences and mouse PrP 135-155 sequences. FIG. 11A depicts sequences of prion chimeras designated GCAB/mouse Chimera No. 1 (SEQ ID NO:64); GCAB/mouse Chimera No. 2 (SEQ ID NO:65); and GCAB/mouse Chimera No. 3 (SEQ ID NO:66). FIG. 11B depicts sequences of prion chimeras designated GCAB/mouse Chimera No. 4 (SEQ ID NO:67); GCAB/mouse Chimera No. 5 (SEQ ID NO:68); and GCAB/mouse Chimera No. 6 (SEQ ID NO:69). Chimeras designated GCAB/mouse (135-155) Chimera No. 1-6 include

sequences derived from GCAB (SEQ ID NO:11); sequences derived from mouse PrP (135-155) (SEQ ID NO:7); and a His tag.

FIGs. 12A – 12D depict exemplary prion chimeras including "P69 control A" non-prion amino acid sequences and human PrP prion amino acid sequences (126-154). FIG. 12A depicts sequences of prion chimeras designated P69A/human Chimera No. 1 (SEQ ID NO:70) and P69A/human Chimera No. 2 (SEQ ID NO:71). FIG. 12B depicts sequences of prion chimeras designated P69A/human Chimera No. 3 (SEQ ID NO:72) and P69A/human Chimera No. 4 (SEQ ID NO:73). FIG. 12C depicts sequences of prion chimeras designated P69A/human Chimera No. 5 (SEQ ID NO:74) and P69A/human Chimera No. 6 (SEQ ID NO:75). FIG. 12D depicts sequences of prion chimeras designated P69A/human Chimera No. 7 (SEQ ID NO:76) and P69A/human Chimera No. 8 (SEQ ID NO:77). Chimeras designated P69A/human 126-154 Chimera No. 1-8 include sequences derived from P69 Control A (SEQ ID NO:8); sequences derived from human PrP (126-154) (SEQ ID NO:12); and a His tag.

FIGs. 13A – 13D depict exemplary prion chimeras including "P69 Control A" non-prion amino acid sequences and mouse PrP prion amino acid sequences (126-154). FIG. 13A depicts sequences of prion chimeras designated P69A/mouse Chimera No. 1 (SEQ ID NO:78) and P69A/mouse Chimera No. 2 (SEQ ID NO:79). FIG. 13B depicts sequences of prion chimeras designated P69A/mouse Chimera No. 3 (SEQ ID NO:80) and P69A/mouse Chimera No. 4 (SEQ ID NO:81). FIG. 13C depicts sequences of prion chimeras designated P69A/mouse Chimera No. 5 (SEQ ID NO:82) and P69A/mouse Chimera No. 6 (SEQ ID NO:83). FIG. 13D depicts sequences of prion chimeras designated P69A/mouse Chimera No. 7 (SEQ ID NO:84) and P69A/mouse Chimera No. 8 (SEQ ID NO:85). Chimeras designated P69A/mouse 126-154 Chimera No. 1-8 include sequences derived from P69 Control A (SEQ ID NO:8); sequences derived from mouse PrP (126-154) (SEQ ID NO:13); and a His tag.

FIGs. 14A – 14D depict exemplary prion chimeras including "P69 control B" non-prion amino acid sequences and human PrP prion amino acid sequences (126-154). FIG. 14A depicts sequences of prion chimeras designated P69B/human Chimera No. 1 (SEQ ID NO:86) and P69B/human Chimera No. 2 (SEQ ID NO:87). FIG. 14B depicts sequences of prion chimeras designated P69B/human Chimera No. 3 (SEQ ID NO:88) and P69B/human Chimera No. 4 (SEQ ID NO:89). FIG. 14C depicts sequences of prion chimeras designated P69B/human Chimera No. 5 (SEQ ID NO:90) and P69B/human Chimera No. 6 (SEQ ID NO:91). FIG. 14D depicts sequences of prion chimeras designated P69B/human Chimera

No. 7 (SEQ ID NO:92) and P69B/human Chimera No. 8 (SEQ ID NO:93). Chimeras designated P69B/human 126-154 Chimera No. 1-8 include sequences derived from P69 Control B (SEQ ID NO:9); sequences derived from human PrP (126-154) (SEQ ID NO:12); and a His tag.

FIGs. 15A – 15D depict exemplary prion chimeras including "P69 Control B" non-prion amino acid sequences and mouse PrP prion amino acid sequences (126-154). FIG. 15A depicts sequences of prion chimeras designated P69B/mouse Chimera No. 1 (SEQ ID NO:94) and P69B/mouse Chimera No. 2 (SEQ ID NO:95). FIG. 15B depicts sequences of prion chimeras designated P69B/mouse Chimera No. 3 (SEQ ID NO:96) and P69B/mouse Chimera No. 4 (SEQ ID NO:97). FIG. 15C depicts sequences of prion chimeras designated P69B/mouse Chimera No. 5 (SEQ ID NO:98) and P69B/mouse Chimera No. 6 (SEQ ID NO:99). FIG. 15D depicts sequences of prion chimeras designated P69B/mouse Chimera No. 7 (SEQ ID NO:100) and P69B/mouse Chimera No. 8 (SEQ ID NO:101). Chimeras designated P69B/mouse 126-154 Chimera No. 1-8 include sequences derived from P69 Control B (SEQ ID NO:9); sequences derived from mouse PrP (126-154) (SEQ ID NO:13); and a His tag.

FIGs. 16A – 16B depict exemplary prion chimeras including "GCA Control A" non-prion sequences and human PrP 126-154 sequences. FIG. 16A depicts sequences of prion chimeras designated GCAA/human Chimera No. 1 (SEQ ID NO:102); GCAA/human Chimera No. 2 (SEQ ID NO:103); and GCAA/human Chimera No. 3 (SEQ ID NO:104). FIG. 16B depicts sequences of prion chimeras designated GCAA/human Chimera No. 4 (SEQ ID NO:105); GCAA/human Chimera No. 5 (SEQ ID NO:106); and GCAA/human Chimera No. 6 (SEQ ID NO:107). Chimeras designated GCAA/human 126-154 Chimera No. 1-6 include sequences derived from GCAA (SEQ ID NO:10); sequences derived from human PrP (126-154) (SEQ ID NO:12); and a His tag.

FIGs. 17A – 17B depict exemplary prion chimeras including "GCA Control A" non-prion sequences and mouse PrP 126-154 sequences. FIG. 17A depicts sequences of prion chimeras designated GCAA/mouse Chimera No. 1 (SEQ ID NO:108); GCAA/mouse Chimera No. 2 (SEQ ID NO:109); and GCAA/mouse Chimera No. 3 (SEQ ID NO:110). FIG. 17B depicts sequences of prion chimeras designated GCAA/mouse Chimera No. 4 (SEQ ID NO:111); GCAA/mouse Chimera No. 5 (SEQ ID NO:112); and GCAA/mouse Chimera No. 6 (SEQ ID NO:113). Chimeras designated GCAA/mouse 126-154 Chimera No. 1-6 include sequences derived from GCAA (SEQ ID NO:10); sequences derived from mouse PrP (126-154) (SEQ ID NO:8); and a His tag.

FIGs. 18A – 18B depict exemplary prion chimeras including "GCA Control B" non-prion sequences and human PrP 126-154 sequences. FIG. 18A depicts sequences of prion chimeras designated GCAB/human Chimera No. 1 (SEQ ID NO:114); GCAB/human Chimera No. 2 (SEQ ID NO:115); and GCAB/human Chimera No. 3 (SEQ ID NO:116). FIG. 18B depicts sequences of prion chimeras designated GCAB/human Chimera No. 4 (SEQ ID NO:117); GCAB/human Chimera No. 5 (SEQ ID NO:118); and GCAB/human Chimera No. 6 (SEQ ID NO:119). Chimeras designated GCAB/human (126-154) Chimera No. 1-6 include sequences derived from GCAB (SEQ ID NO:11); sequences derived from human PrP (126-154) (SEQ ID NO:12); and a His tag.

FIGs. 19A – 19B depict exemplary prion chimeras including "GCA Control B" non-prion sequences and mouse PrP 126-154 sequences. FIG. 19A depicts sequences of prion chimeras designated GCAB/mouse Chimera No. 1 (SEQ ID NO:120); GCAB/mouse Chimera No. 2 (SEQ ID NO:121); and GCAB/mouse Chimera No. 3 (SEQ ID NO:122). FIG. 19B depicts sequences of prion chimeras designated GCAB/mouse Chimera No. 4 (SEQ ID NO:123); GCAB/mouse Chimera No. 5 (SEQ ID NO:124); and GCAB/mouse Chimera No. 6 (SEQ ID NO:125). Chimeras designated GCAB/mouse (126-154) Chimera No. 1-6 include sequences derived from GCAB (SEQ ID NO:11); sequences derived from mouse PrP (126-154) (SEQ ID NO:13); and a His tag.

FIGs. 20A – 20H depict exemplary DNA constructs and prion chimeras encoded by these constructs, including a tpa leader sequence and mouse prion sequences. Human prion sequences may be used in these molecules. FIG. 20A depicts the DNA construct designated Tpa.p69.mprpf1 (SEQ ID NO:155) and the prion chimera encoded by Tpa.p69.mprpf1 (SEQ ID NO:126). FIG. 20B depicts the DNA construct designated Tpa.p69.mprpf2 (SEQ ID NO:156) and the prion chimera encoded by Tpa.p69.mprpf2 (SEQ ID NO:127). FIG. 20C depicts the DNA construct designated Tpa.p69.mprpf3 (SEQ ID NO:157) and the prion chimera encoded by Tpa.p69.mprpf3 (SEQ ID NO:128). FIG. 20D depicts the DNA construct designated Tpa.p69.mprpf4 (SEQ ID NO:158) and the prion chimera encoded by Tpa.p69.mprpf4 (SEQ ID NO:129). FIG. 20E depicts the DNA construct designated Tpa.p69.mprpf5 (SEQ ID NO:159) and the prion chimera encoded by Tpa.p69.mprpf5 (SEQ ID NO:130). FIG. 20F depicts the DNA construct designated and Tpa.p69.mprpf6 (SEQ ID NO:160) and the prion chimera encoded by Tpa.p69.mprpf6 (SEQ ID NO:131). FIG. 20G depicts the DNA construct designated Tpa.p69.mprpf7 (SEQ ID NO:161) and the prion chimera encoded by Tpa.p69.mprpf7 (SEQ ID NO:132). FIG. 20H depicts the DNA

construct designated Tpa.p69.mprpf8 (SEQ ID NO:162) and the prion chimera encoded by Tpa.p69.mprpf8 (SEQ ID NO:133). The constructs designated tpa.p69.mprpf1 through tpa.p69.mprpf8 include sequences encoding a tpa leader peptide sequence (SEQ ID NO:154); P69 non-prion sequences and prion sequences (135-155, SEQ ID NO:7). The prion sequences are 3' to the P69-encoding sequences in the construct and C-terminal to the P69 sequences in the chimera.

FIGs. 21A – 21H depict exemplary DNA constructs and prion chimeras encoded by these constructs, including a tpa leader sequence and mouse prion sequences. Human prion sequences may be used in these molecules. FIG. 21A depicts the DNA construct designated Tpa.mprpf1.69 (SEQ ID NO:163) and the chimera encoded by Tpa.mprpf1.69 (SEQ ID NO:134). FIG. 21B depicts the DNA construct designated Tpa.mprpf2.69 (SEQ ID NO:164) and the prion chimera encoded by Tpa.mprpf2.69 (SEQ ID NO:135). FIG. 21C depicts the DNA construct designated Tpa.mprpf3.69 (SEQ ID NO:165) and the prion chimera encoded by Tpa.mprpf3.69 (SEQ ID NO:136). FIG. 21D depicts the DNA construct designated Tpa.mprpf4.69 (SEQ ID NO:166) and the prion chimera encoded by Tpa.mprpf4.69 (SEQ ID NO:137). FIG. 21E depicts the DNA construct designated Tpa.mprpf5.69 (SEQ ID NO:167) and the prion chimera encoded by Tpa.mprpf5.69 (SEQ ID NO:138). FIG. 21F depicts the DNA construct designated Tpa.mprpf6.69 (SEQ ID NO:168) and the prion chimera encoded by and Tpa.mprpf6.69 (SEQ ID NO:139). FIG. 21G depicts the DNA construct designated Tpa.mprpf7.69 (SEQ ID NO:169) and the prion chimera encoded by Tpa.mprpf7.69 (SEQ ID NO:140). FIG. 21H depicts the DNA construct designated Tpa.mprpf8.69 (SEQ ID NO:170) and the prion chimera encoded by and Tpa.mprpf869 (SEQ ID NO:141). The constructs designated tpa.mprpf1.69 through tpa.mprpf8.69 include sequences encoding a tpa leader peptide sequence (SEQ ID NO:154); P69 non-prion sequences and prion sequences (135-155, SEQ ID NO:7). The prion sequences are 5' to the P69-encoding sequences in the construct and N-terminal to the P69 sequences in the chimera.

FIGS. 22A – 22C depict exemplary DNA constructs and prion chimeras encoded by these constructs, including a tpa leader sequence and mouse prion sequences. Human prion sequences may be used in these molecules. FIG. 22A depicts DNA constructs designated Tpa.mprpf1.gca (SEQ ID NO:171); the prion chimera encoded by Tpa.mprpf1.gca (SEQ ID NO:142); Tpa.mprpf2.gca (SEQ ID NO:172); and the prion chimera encoded by Tpa.mprpf2.gca (SEQ ID NO:143). FIG. 22B depicts DNA constructs designated Tpa.mprpf3.gca (SEQ ID NO:173); the prion chimera encoded by Tpa.mprpf3.gca (SEQ ID NO:144); Tpa.mprpf4.gca (SEQ ID NO:174); and the prion chimera encoded by

Tpa.mprpf4.gca (SEQ ID NO:145). FIG. 22C depicts DNA constructs designated Tpa.mprpf5.gca (SEQ ID NO:175); the prion chimera encoded by Tpa.mprpf5.gca (SEQ ID NO:146); Tpa.mprpf6.gca (SEQ ID NO:176); and the prion chimera encoded by Tpa.mprpf6.gca (SEQ ID NO:147). The constructs designated Tpa.mprpf1.gca through Tpa.mprpf6.gca include sequences encoding a tpa leader peptide sequence (SEQ ID NO:154); GCA non-prion sequences and prion sequences (135-155, SEQ ID NO:7). The prion sequences are 5' to the GCA-encoding sequences in the construct and N-terminal to the GCA non-prion sequences in the chimera.

FIGS. 23A – 23C depict exemplary DNA constructs and prion chimeras encoded by these constructs, including a tpa leader sequence and mouse prion sequences. Human prion sequences may be used in these molecules. FIG. 23A depicts the DNA construct designated Tpa.gca.mprpf1 (SEQ ID NO:177); the chimera encoded by Tpa.gca.mprpf1 (SEQ ID NO:148); the DNA construct designated Tpa.gca.mprpf2 (SEQ ID NO:178); and the chimera encoded by Tpa.gca.mprpf2 (SEQ ID NO:149). FIG.23B depicts the DNA construct designated Tpa.gac.mprpf3 (SEQ ID NO:179); the chimera encoded by Tpa.gca.mprpf3 (SEQ ID NO:150); the DNA construct designated Tpa.gca.mprpf4 (SEQ ID NO:180); and the chimera encoded by Tpa.gca.mprpf4 (SEQ ID NO:151). FIG. 23C depicts the DNA construct designated Tpa.gca.mprp5 (SEQ ID NO:181); the chimera encoded by Tpa.gca.mprpf5 (SEQ ID NO:152); the DNA construct designated Tpa.gca.mprp6 (SEQ ID NO:182); and the chimera encoded by Tpa.gca.mprpf6 (SEQ ID NO:153). The constructs designated Tpa.gca.mprpf1 through Tpa.gca.mprpf6 include sequences encoding a tpa leader peptide sequence (SEQ ID NO:154); GCA non-prion sequences and mouse prion sequences (135-155, SEQ ID NO:7). The prion sequences are 3' to the GCA-encoding sequences in the constructs and C-terminal to the GCA non-prion sequences in the chimeras.

FIG. 24 depicts an exemplary tpa leader amino acid sequence of 25 amino acids in length (SEQ ID NO:154) and an exemplarly GCA leader sequence M M F N K (SEQ ID NO:209) that was removed from GCA full length (SEQ ID NO:4) and GCA Control B (SEQ ID NO:11) to make GCA full length no leader (SEQ ID NO:183) and GCA Control B No Leader (SEQ ID NO:184), respectively.

FIGs. 25A – 25B depict exemplary prion chimeras including "GCA Control B no leader" non-prion sequences (SEQ ID NO:184) and human PrP 135-155 sequences. FIG. 25A depicts sequences of prion chimeras designated GCABnoL/human Chimera No. 1 (SEQ ID NO:185); GCABnoL/human Chimera No. 2 (SEQ ID NO:186); and GCABnoL/human Chimera No. 3 (SEQ ID NO:187). FIG. 25B depicts sequences of prion chimeras designated

GCABnoL/human Chimera No. 4 (SEQ ID NO:188); GCABnoL/human Chimera No. 5 (SEQ ID NO:189); and GCABnoL/human Chimera No. 6 (SEQ ID NO:190). Chimeras designated GCABnoL/human (135-155) Chimera No. 1-6 include sequences derived from GCABnoL (SEQ ID NO:184); sequences derived from human PrP (135-155) (SEQ ID NO:6); and a His tag.

FIGs. 26A – 26B depict exemplary prion chimeras including "GCA Control B no leader" non-prion sequences and mouse PrP 135-155 sequences. FIG. 26A depicts sequences of prion chimeras designated GCABnoL/mouse Chimera No. 1 (SEQ ID NO:191); GCABnoL/mouse Chimera No. 2 (SEQ ID NO:192); and GCABnoL/mouse Chimera No. 3 (SEQ ID NO:193). FIG. 26B depicts sequences of prion chimeras designated GCABnoL/mouse Chimera No. 4 (SEQ ID NO:194); GCABnoL/mouse Chimera No. 5 (SEQ ID NO:195); and GCABnoL/mouse Chimera No. 6 (SEQ ID NO:196). Chimeras designated GCABnoL/mouse (135-155) Chimera No. 1-6 include sequences derived from GCABnoL (SEQ ID NO:184); sequences derived from mouse PrP (135-155) (SEQ ID NO:7); and a His tag.

FIGs. 27A – 27B depict exemplary prion chimeras including "GCA Control B no leader" non-prion sequences and human PrP 126-154 sequences. FIG. 27A depicts sequences of prion chimeras designated GCABnoL/human Chimera No. 1 (SEQ ID NO:197); GCABnoL/human Chimera No. 2 (SEQ ID NO:198); and GCABnoL/human Chimera No. 3 (SEQ ID NO:199). FIG. 27B depicts sequences of prion chimeras designated GCABnoL/human Chimera No. 4 (SEQ ID NO:200); GCABnoL/human Chimera No. 5 (SEQ ID NO:201); and GCABnoL/human Chimera No. 6 (SEQ ID NO:202). Chimeras designated GCABnoL/human (126-154) Chimera No. 1-6 include sequences derived from GCABnoL (SEQ ID NO:184); sequences derived from human PrP (126-154) (SEQ ID NO:12); and a His tag.

FIGs. 28A – 28B depict exemplary prion chimeras including "GCA Control B no leader" non-prion sequences and mouse PrP 126-154 sequences. FIG. 28A depicts sequences of prion chimeras designated GCABnoL/mouse Chimera No. 1 (SEQ ID NO:203); GCABnoL/mouse Chimera No. 2 (SEQ ID NO:204); and GCABnoL/mouse Chimera No. 3 (SEQ ID NO:205). FIG. 28B depicts sequences of prion chimeras designated GCABnoL/mouse Chimera No. 4 (SEQ ID NO:206); GCABnoL/mouse Chimera No. 5 (SEQ ID NO:207); and GCABnoL/mouse Chimera No. 6 (SEQ ID NO:208). Chimeras designated GCABnoL/mouse (126-154) Chimera No. 1-6 include sequences derived from GCABnoL

(SEQ ID NO:184); sequences derived from mouse PrP (126-154) (SEQ ID NO:13); and a His tag.

BRIEF DESCRIPTION OF SEQUENCE ID NOS.

The following SEQ ID NOS are used in the application and the figures.

SEQ ID NO. 1 represents a full-length amino acid sequence of the human prion protein.

SEQ ID NO. 2 represents a full-length amino acid sequence of the mouse prion protein.

SEQ ID NO. 3 represents an amino acid sequence of P69.

SEQ ID NO. 4 represents an amino acid sequence of GCA.

SEQ ID NO. 5 is the following amino acid sequence that represents a histidine tag: G G H H H H H H.

SEQ ID NO. 6 represents an amino acid sequence of a human PrP fragment (135 – 155).

SEQ ID NO. 7 represents an amino acid sequence of a mouse PrP fragment (135 – 155).

SEQ ID NO. 8 represents an amino acid sequence of P69 Control A.

SEQ ID NO. 9 represents an amino acid sequence of P69 Control B.

SEQ ID NO. 10 represents an amino acid sequence of GCA Control A.

SEQ ID NO. 11 represents an amino acid sequence of GCA Control B.

SEQ ID NO. 12 represents an amino acid sequence of a human PrP fragment (126 – 154).

SEQ ID NO. 13 represents an amino acid sequence of a mouse PrP fragment (126 – 154).

SEQ ID NOS. 14 - 21 represent examples of prion chimeras comprising human PrP (135 – 155) – P69 Control A – His Tag.

SEQ ID NOS. 22 – 29 represent examples of prion chimeras comprising mouse PrP (135 – 155) – P69 Control A – His Tag.

SEQ ID NOS. 30 – 37 represent examples of prion chimeras comprising a His Tag – P69 Control B – Hu PrP (135 – 155).

SEQ ID NOS. 38 – 45 represent examples of prion chimeras comprising a His Tag – P69 Control B – Mo PrP (135 – 155).

SEQ ID NOS. 46 – 51 represent examples of prion chimeras comprising Hu PrP (135 – 155) – GCA Control A – His Tag.

SEQ ID NOS. 52 – 57 represent examples of prion chimeras comprising Mo PrP (135 – 155) – GCA Control A – His Tag.

SEQ ID NOS. 58 – 63 represent examples of prion chimeras comprising His Tag – GCA Control B – Hu PrP (135 – 155).

SEQ ID NOS. 64 – 69 represent examples of prion chimeras comprising His Tag – GCA Control B – Mo PrP (135 – 155).

SEQ ID NOS. 70 - 77 represent examples of prion chimeras comprising human PrP (126 – 154) – P69 Control A – His Tag.

SEQ ID NOS. 78 – 85 represent examples of prion chimeras comprising mouse PrP (126 – 154) – P69 Control A – His Tag.

SEQ ID NOS. 86 – 93 represent examples of prion chimeras comprising a His Tag – P69 Control B – Hu PrP (126 – 154).

SEQ ID NOS. 94 – 101 represent examples of prion chimeras comprising a His Tag – P69 Control B – Mo PrP (126 – 154).

SEQ ID NOS. 102 – 107 represent examples of prion chimeras comprising Hu PrP (126 – 154) – GCA Control A – His Tag.

SEQ ID NOS. 108 – 113 represent examples of prion chimeras comprising Mo PrP (126 – 154) – GCA Control A – His Tag.

SEQ ID NOS. 114 – 119 represent examples of prion chimeras comprising His Tag – GCA Control B – Hu PrP (126 – 154).

SEQ ID NOS. 120 – 125 represent examples of prion chimeras comprising His Tag – GCA Control B – Mo PrP (126 – 154).

SEQ ID NOS. 126 – 133 represent examples of prion chimeras encoded by DNA constructs where the chimeras include a tpa signal sequence and P69 non-prion sequences.

SEQ ID NOS. 134 – 141 represent examples of prion chimeras encoded by DNA constructs where the chimeras include a tpa signal sequence, prion sequences and P69 non-prion sequences.

SEQ ID NOS. 142 – 147 represent examples of prion chimeras encoded by DNA constructs where the chimeras include a tpa signal sequence, prion sequences and GCA non-prion sequences.

SEQ ID NOS. 148 – 153 represent examples of prion chimeras encoded by DNA constructs where the chimeras include a tpa signal sequence, prion sequences and GCA non-prion sequences.

SEQ ID NO:154 is an exemplary tpa leader sequence.

SEQ ID NOs: 155 – 170 represent examples of DNA constructs encoding prion chimera polypeptides that include a tpa signal sequence, prion sequences and P69 non-prion sequences.

SEQ ID NOs: 171 – 182 represent examples of DNA constructs encoding prion chimera polypeptides that include a tpa signal sequence, prion sequences and GCA non-prion sequences.

SEQ ID NO:183 represents an amino acid sequence of GCA without the leader sequence.

SEQ ID NO:184 represents an amino acid sequence of GCA Control B without the leader sequence.

SEQ ID NOS. 185 – 190 represent examples of prion chimeras comprising His Tag – GCA Control B no leader – Hu PrP (135 – 155).

SEQ ID NOS. 191 – 196 represent examples of prion chimeras comprising His Tag – GCA Control B no leader – Mo PrP (135 – 155).

SEQ ID NOS. 197 – 202 represent examples of prion chimeras comprising His Tag – GCA Control B no leader – Hu PrP (126 – 154).

SEQ ID NOS. 203 – 208 represent examples of prion chimeras comprising His Tag – GCA Control B no leader – Mo PrP (126 – 154).

SEQ ID NO. 209 is the following amino acid sequence that represents a GCA leader sequence: M M F N K.

DETAILED DESCRIPTION

The invention relates to prion chimeras, polynucleotides encoding prion chimeras, methods of generating antibodies using prion chimeras and polynucleotides encoding prion chimeras, as well as to antibodies specific for prion chimeras and polynucleotides encoding these antibodies. The invention further relates to methods of using such antibodies, chimeras and/or polynucleotides to detect the presence of pathogenic prions, for example in a biological sample. In addition, the invention further relates to methods of using such antibodies, chimeras and/or polynucleotides as a component in a therapeutic or prophylactic vaccine.

The prion chimeras (and polynucleotides encoding these chimeras) used in the invention typically comprise a polypeptide comprising a first segment and a second segment. The first segment comprises a prion polypeptide (or fragment thereof) and the second segment comprises a non-prion protein (or fragment thereof). Typically, the prion polypeptide comprises an amino acid sequence of a prion protein that can adapt the β-helical conformation of a pathogenic prion protein. Preferably, the prion component of the chimera adapts a β-helix conformation within the chimera. Similarly, the non-prion protein typically comprises at least a fragment of a non-prion β-helical protein, for example pertactin or GCA. The non-prion protein used in the chimera generally does not contain a portion of the amino acids of the non-prion protein that adapt the β-helix conformation.

The practice of the present invention will employ, unless otherwise indicated, conventional methods of chemistry, biochemistry, molecular biology, immunology and pharmacology, within the skill of the art. Such techniques are explained fully in the literature. See, e.g., *Remington's Pharmaceutical Sciences*, 18th Edition (Easton, Pennsylvania: Mack Publishing Company, 1990); *Methods In Enzymology* (S. Colowick and N. Kaplan, eds., Academic Press, Inc.); and *Handbook of Experimental Immunology*, Vols. I-IV (D.M. Weir and C.C. Blackwell, eds., 1986, Blackwell Scientific Publications); Sambrook, et al., *Molecular Cloning: A Laboratory Manual* (2nd Edition, 1989); *Handbook of Surface and Colloidal Chemistry* (Birdi, K.S. ed., CRC Press, 1997); *Short Protocols in Molecular Biology*, 4th ed. (Ausubel et al. eds., 1999, John Wiley & Sons); *Molecular Biology Techniques: An Intensive Laboratory Course*, (Ream et al., eds., 1998, Academic Press); *PCR (Introduction to Biotechniques Series)*, 2nd ed. (Newton & Graham eds., 1997, Springer Verlag); Peters and Dalrymple, *Fields Virology* (2d ed), Fields et al. (eds.), B.N. Raven Press, New York, NY.

It is understood that the prion chimeras, antibodies and methods of this invention are not limited to particular formulations or process parameters as such may, of course, vary. It is also to be understood that the terminology used herein is for the purpose of describing particular embodiments of the invention only, and is not intended to be limiting.

All publications, patents and patent applications cited herein are hereby incorporated by reference in their entirety.

I. Definitions

In order to facilitate an understanding of the invention, selected terms used in the application will be discussed below.

The terms "prion", "PrP protein" and "PrP" are used interchangeably herein to refer to both the pathogenic particle form PrP^{SC} associated with disease state (spongiform encephalopathies) in humans and animals and the nonpathogenic form PrP^{C} which may, under appropriate conditions, may be converted to the pathogenic PrP^{SC} form. Prions are naturally produced in a wide variety of mammalian species, including human, sheep, cattle, and mice. A representative amino acid sequence of a human prion protein is set forth as SEQ ID NO:1. A representative amino acid sequence of a mouse prion protein is set forth as SEQ ID. NO:2. Variants of the prion proteins, including deletions, additions and other mutations are known in the art. The pathogenic conformation of the prion protein typically includes at least one region that can adapt a β-helical conformation (referred to as a "β-helical region"). For example, in Figure 1A (SEQ ID NO:1), helical regions occur at approximately amino acid residues 144 to 153 and at approximately residues 173 to 194, numbered relative to the human PrP shown in SEQ ID NO:1. Beta-strand regions occur at approximately amino acid 129 to 133 and at approximately amino acid residues 160 to 163, numbered relative to the human PrP shown in SEQ ID NO:1. Pathogenic forms may or may not be infectious. As used herein, the term "pathogenic" may mean that the protein actually causes the disease or it may simply mean that the protein is associated with the disease and therefore is present when the disease is present. Thus, a pathogenic protein as used in connection with this disclosure is not necessarily a protein that is the specific causative agent of a disease.

Furthermore, a "prion protein" or "conformational disease protein" as defined herein is not limited to a polypeptide having the exact sequence to those described herein. It is readily apparent that the terms encompass conformational disease proteins (*e.g.*, prion proteins) from any of the identified or unidentified species or disease. Descriptions of structural features are given herein with reference to a human and/or mouse sequence, presented in the Figures. One of ordinary skill in the art in view of the teachings of the present disclosure and the art can determine corresponding regions in other prion proteins, using for example, sequence comparison programs (*e.g.*, BLAST and others described herein) or identification and alignment of structural features (*e.g.*, a program such as the "ALB" program described herein that can identify β-helix forming regions).

The term "PrP gene" is used herein to describe any genetic material that expresses proteins including known polymorphisms and pathogenic mutations. The term "PrP gene" refers generally to any gene of any species that encodes any form of a PrP protein. Some

commonly known PrP sequences are described in Gabriel et al., Proc. Natl. Acad. Sci. USA 89:9097-9101 (1992), and U.S. Pat. Nos. 5,565,186; 5,763,740; 5,792,901; and WO97/04814, incorporated herein by reference to disclose and describe such sequences. The PrP gene can be from any animal, including the "host" and "test" animals described herein and any and all polymorphisms and mutations thereof, it being recognized that the terms include other such PrP genes that are yet to be discovered. The protein expressed by such a gene can assume either a PrP^{C} (non-disease) or PrP^{SC} (disease) form.

By "geometry" or "tertiary structure" of a polypeptide or protein is meant the overall 3-D configuration of the protein. As described herein, the geometry can be determined, for example, by crystallography studies or by using various programs or algorithms that predict the geometry based on interactions between the amino acids making up the primary and secondary structures.

"β-helix" as used herein refers to a protein conformation comprising a helical structure that contains highly ordered, stacked side chains on both the inside and outside of the helix. This side-chain stacking adds to the rigid geometry of the β-helices, which tend to have planar sides and essentially no interstrand twist. β-helices include both left- and right-handed structures. The number of residues per β-helical turn for left-handed helices averages about 18 residues per helical turn. The number of residues per β-helical turn for right-handed helices can vary substantially, but averages about 24 residues per helical turn. *See* Wille, et al., "Structural Studies of the Scrapie Prion Protein by Electron Crystallography", *Proc. Natl. Acad. Sci. USA*, 99 (6): 3563-3568 (2002) and T.E. Creighton, Proteins: Structures and Molecular Properties (W.H. Freeman and Company, 1993); and A.L. Lehninger, Biochemistry (Worth Publishers, Inc., 1975).

"Prion-related disease" as used herein refers to a disease caused in whole or in part by a pathogenic prion particle (PrP^{Sc}). Prion-related diseases include scrapie, bovine spongiform encephalopathies (BSE), mad cow disease, feline spongiform encephalopathies, kuru, Creutzfeldt-Jakob Disease (CJD), Gerstmann-Strassler-Scheinker Disease (GSS), and fatal familial insomnia (FFI).

The terms "polypeptide", "protein" and "amino acid sequence" as used herein generally refer to a polymer of amino acid residues and are not limited to a minimum length of the product. Thus, peptides, oligopeptides, dimers, multimers, and the like, are included within the definition. Both full-length proteins and fragments thereof are encompassed by the definition. Minimum fragments of polypeptides useful in the invention can be at least 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 20, 25 or even longer amino acids. Where the polypeptide

comprises a β-helical region of a prion protein, the polypeptide preferably comprises enough amino acids to adapt a β-helical conformation. Typically, polypeptides useful in this invention can have a maximum length suitable for the intended application. Generally, the maximum length is not critical and can easily be selected by one skilled in the art.

Reference to polypeptides and the like also includes derivatives of the amino acid sequences of the invention. A first polypeptide is "derived from" a second polypeptide if it is (i) encoded by a first polynucleotide derived from a second polynucleotide, or (ii) displays sequence identity to the second polypeptides as described herein. Such derivatives can include postexpression modifications of the polypeptide, for example, glycosylation, acetylation, phosphorylation, and the like. Amino acid derivatives can also include modifications to the native sequence, such as deletions, additions and substitutions (generally conservative in nature), so long as the protein maintains the desired activity. These modifications may be deliberate, as through site-directed mutagenesis, or may be accidental, such as through mutations of hosts that produce the proteins or errors due to PCR amplification. Furthermore, modifications may be made that have one or more of the following effects: reducing toxicity; facilitating cell processing (*e.g.*, secretion, antigen presentation, etc.); and facilitating presentation to B-cells and/or T-cells. Similarly, if the polypeptides are to be used for diagnostic purposes, such capability must be retained.

A "fragment" as used herein refers to a polypeptide consisting of only a part of the intact full-length polypeptide sequence and structure as found in nature. For instance, a fragment can include a C-terminal deletion and/or an N-terminal deletion of a protein. Typically, the fragment retains one, some or all of the functions of the full-length polypeptide sequence from which it is derived.

A "recombinant" protein is a protein that has been prepared by recombinant DNA techniques as described herein. In general, the gene of interest is cloned and then expressed in transformed organisms, as described further below. The host organism expressed the foreign gene to produce the protein under expression conditions.

The phrase "prion chimera" as used herein generally refers to a polypeptide comprising at least two different segments (*e.g.*, amino acid sequences obtained from different sources/proteins). At least one segment comprises a prion polypeptide (or fragment thereof) or a sequence derived from a prion, while the other segment comprises a non-prion polypeptide or a sequence derived from a non-prion polypeptide (*e.g.*, a sequence obtained from a β-helical protein). Prion chimeras may be the form of a fusion protein and may include additional elements, for example a tagging sequence (*e.g.*, a His tagging sequence

that may serve to facilitate purification and increase solubility of the chimera). The different segments may be co-linear (N- and/or C-terminal to each other) or, alternatively, one segment may be inserted into another segment so that it is flanked by the sequences of the other segment.

A "non-prion protein" as used herein refers to any protein that is not a prion polypeptide. A "non-prion β-helical protein" as used herein refers to a non-prion protein that contains an amino acid sequence that can adapt a β-helical conformation. Such β-helical proteins include proteins that contain right-handed and/or left-handed β-helices. Non-limiting examples of right handed beta-helix proteins include pectate lyase, pectin lyase, galacturonase, chondroitinase B, pectin methylesterase, P22 tailspike protein, virulence factor P69 pertactin, insect cysteine-rich antifreeze protein, cell-division inhibitor MinC, alpha subunit of glutamate synthase (*e.g.,* C-terminal domain) and C-terminal domain of adenylylcyclase associated protein. Non-limiting examples of left handed beta-helix proteins include UDP N-acetylglucosamine acyltransferase, galactoside acetyltransferase, xenobiotic acetyltransferase, tetrahydrodipicolinate-N-succinlytransferase, THDP-succinlytransferase, DapD N-acetylglucosamine 1-phosphate uridyltransferase GlmU, and C-terminal domain of carbonic anhydrase. In certain embodiments, the non-prion protein comprises sequence derived from γ carbonic anhydrase (left handed beta-helical protein) while in other embodiments, the non-prion polypeptide comprises sequence derived from pertactin (right handed beta-helical protein).

The term "polynucleotide", as known in the art, generally refers to a nucleic acid molecule. A "polynucleotide" can include both double- and single-stranded sequences and refers to, but is not limited to, cDNA from viral, prokaryotic or eukaryotic MRNA, genomic RNA and DNA sequences from viral (e.g. RNA and DNA viruses and retroviruses) or prokaryotic DNA, and especially synthetic DNA sequences. The term also captures sequences that include any of the known base analogs of DNA and RNA, and includes modifications such as deletions, additions and substitutions (generally conservative in nature), to the native sequence, so long as the nucleic acid molecule encodes a therapeutic or antigenic protein. These modifications may be deliberate, as through site-directed mutagenesis, or may be accidental, such as through mutations of hosts that produce the antigens. Modifications of polynucleotides may have any number of effects including, for example, facilitating expression of the polypeptide product in a host cell.

A polynucleotide can encode a biologically active (e.g., immunogenic or therapeutic) protein or polypeptide. Depending on the nature of the polypeptide encoded by the

polynucleotide, a polynucleotide can include as little as 10 nucleotides, e.g., where the polynucleotide encodes an antigen.

Where the polynucleotide encodes a prion (or fragment thereof), preferably the polynucleotide is long enough to encode for a β-helical region of the prion. Typically, where the polynucleotide encodes a β-helical region of a prion, the polynucleotide includes at least 18, 19, 20, 21, 22, 23, 24, 25, 30 or even more amino acids.

A "polynucleotide coding sequence" or a sequence that "encodes" a selected polypeptide, is a nucleic acid molecule that is transcribed (in the case of DNA) and translated (in the case of mRNA) into a polypeptide *in vivo* when placed under the control of appropriate regulatory sequences (or "control elements"). The boundaries of the coding sequence are determined by a start codon at the 5' (amino) terminus and a translation stop codon at the 3' (carboxy) terminus. Exemplary coding sequences are the modified viral polypeptide-coding sequences of the present invention. A transcription termination sequence may be located 3' to the coding sequence. Typical "control elements", include, but are not limited to, transcription regulators, such as promoters, transcription enhancer elements, transcription termination signals, and polyadenylation sequences; and translation regulators, such as sequences for optimization of initiation of translation, e.g., Shine-Dalgarno (ribosome binding site) sequences, Kozak sequences (i.e., sequences for the optimization of translation, located, for example, 5' to the coding sequence), leader sequences (heterologous or native), translation initiation codon (e.g., ATG), and translation termination sequences. In certain embodiments, one or more translation regulation or initiation sequences (e.g., the leader sequence) are derived from wild-type translation initiation sequences, i.e., sequences that regulate translation of the coding region in their native state. Wild-type leader sequences that have been modified, using the methods described herein, also find use in the present invention. Native or modified leader sequences can be from any source, for example other sources (*e.g.*, wild-type or modified tpa leader sequence exemplified herein and/or leader sequences derived from prion-encoding polynucleotides). Promoters can include inducible promoters (where expression of a polynucleotide sequence operably linked to the promoter is induced by an analyte, cofactor, regulatory protein, etc.), repressible promoters (where expression of a polynucleotide sequence operably linked to the promoter is induced by an analyte, cofactor, regulatory protein, etc.), and constitutive promoters.

A "nucleic acid" molecule can include, but is not limited to, prokaryotic sequences, eukaryotic mRNA, cDNA from eukaryotic mRNA, genomic DNA sequences from eukaryotic

(e.g., mammalian) DNA, and even synthetic DNA sequences. The term also captures sequences that include any of the known base analogs of DNA and RNA.

"Operably linked" refers to an arrangement of elements wherein the components so described are configured so as to perform their usual function. Thus, a given promoter operably linked to a coding sequence is capable of effecting the expression of the coding sequence when the proper enzymes are present. The promoter need not be contiguous with the coding sequence, so long as it functions to direct the expression thereof. Thus, for example, intervening untranslated yet transcribed sequences can be present between the promoter sequence and the coding sequence and the promoter sequence can still be considered "operably linked" to the coding sequence.

A "recombinant" nucleic acid molecule as used herein to describe a nucleic acid molecule means a polynucleotide of genomic, cDNA, semisynthetic, or synthetic origin which, by virtue of its origin or manipulation: (1) is not associated with all or a portion of the polynucleotide with which it is associated in nature; and/or (2) is linked to a polynucleotide other than that to which it is linked in nature. The term "recombinant" as used with respect to a protein or polypeptide means a polypeptide produced by expression of a recombinant polynucleotide. "Recombinant host cells," "host cells," "cells," "cell lines," "cell cultures," and other such terms denoting prokaryotic microorganisms or eukaryotic cell lines cultured as unicellular entities, are used interchangeably, and refer to cells which can be, or have been, used as recipients for recombinant vectors or other transfer DNA, and include the progeny of the original cell which has been transfected. It is understood that the progeny of a single parental cell may not necessarily be completely identical in morphology or in genomic or total DNA complement to the original parent, due to accidental or deliberate mutation. Progeny of the parental cell which are sufficiently similar to the parent to be characterized by the relevant property, such as the presence of a nucleotide sequence encoding a desired peptide, are included in the progeny intended by this definition, and are covered by the above terms.

By "isolated" is meant, when referring to a polynucleotide or a polypeptide, that the indicated molecule is separate and discrete from the whole organism with which the molecule is found in nature or, when the polynucleotide or polypeptide is not found in nature, is sufficiently free of other biological macromolecules so that the polynucleotide or polypeptide can be used for its intended purpose.

"Antibody" as known in the art includes one or more biological moieties that, through chemical or physical means, can bind to or associate with an epitope of a polypeptide of

interest. The antibodies of the invention specifically bind to pathogenic prion conformations. The term "antibody" includes antibodies obtained from both polyclonal and monoclonal preparations, as well as the following: hybrid (chimeric) antibody molecules (see, for example, Winter et al. (1991) *Nature* 349: 293-299; and U.S. Patent No. 4,816,567; $F(ab')_2$ and F(ab) fragments; F_v molecules (non-covalent heterodimers, see, for example, Inbar et al. (1972) *Proc Natl Acad Sci USA* 69:2659-2662; and Ehrlich et al. (1980) *Biochem* 19:4091-4096); single-chain Fv molecules (sFv) (see, for example, Huston et al. (1988) *Proc Natl Acad Sci USA* 85:5897-5883); dimeric and trimeric antibody fragment constructs; minibodies (see, e.g., Pack et al. (1992) *Biochem* 31:1579-1584; Cumber et al. (1992) *J Immunology* 149B: 120-126); humanized antibody molecules (see, for example, Riechmann et al. (1988) *Nature* 332:323-327; Verhoeyan et al. (1988) *Science* 239:1534-1536; and U.K. Patent Publication No. GB 2,276,169, published 21 September 1994); and, any functional fragments obtained from such molecules, wherein such fragments retain immunological binding properties of the parent antibody molecule. The term "antibody" further includes antibodies obtained through non-conventional processes, such as phage display.

As used herein, the term "monoclonal antibody" refers to an antibody composition having a homogeneous antibody population. The term is not limited regarding the species or source of the antibody, nor is it intended to be limited by the manner in which it is made. Thus, the term encompasses antibodies obtained from murine hybridomas, as well as human monoclonal antibodies obtained using human rather than murine hybridomas. See, e.g., Cote, et al. *Monoclonal Antibodies and Cancer Therapy*, Alan R. Liss, 1985, p 77.

If polyclonal antibodies are desired, a selected mammal (e.g., mouse, rabbit, goat, horse, etc.) is generally immunized with an immunogenic composition (*e.g.*, including a prion chimera or polynucleotide encoding a prion chimera that are expressed in the animal). Serum from the immunized animal is collected and treated according to known procedures. If serum containing polyclonal antibodies to the prion chimera contains antibodies to other antigens, the polyclonal antibodies can be purified by immunoaffinity chromatography. Techniques for producing and processing polyclonal antisera are known in the art, see for example, Mayer and Walker, eds. (1987) IMMUNOCHEMICAL METHODS IN CELL AND MOLECULAR BIOLOGY (Academic Press, London).

One skilled in the art can also readily produce monoclonal antibodies directed against prion chimeras described herein. The general methodology for making monoclonal antibodies by hybridomas is well known. Immortal antibody-producing cell lines can be created by cell fusion, and also by other techniques such as direct transformation of B-lymphocytes with

oncogenic DNA, or transfection with Epstein-Barr virus. See, e.g., M. Schreier et al. (1980) HYBRIDOMA TECHNIQUES; Hammerling et al. (1981), MONOCLONAL ANTIBODIES AND T-CELL HYBRIDOMAS; Kennett et al. (1980) MONOCLONAL ANTIBODIES; see also, U.S. Pat. Nos. 4,341,761; 4,399,121; 4,427,783; 4,444,887; 4,466,917; 4,472,500; 4,491,632; and 4,493,890.

As used herein, a "single domain antibody" (dAb) is an antibody that is comprised of an HL domain, which binds specifically with a designated antigen. A dAb does not contain a VL domain, but may contain other antigen binding domains known to exist to antibodies, for example, the kappa and lambda domains. Methods for preparing dabs are known in the art. See, for example, Ward et al, Nature 341: 544 (1989).

Antibodies can also be comprised of VH and VL domains, as well as other known antigen binding domains. Examples of these types of antibodies and methods for their preparation and known in the art (see, e.g., U.S. Pat. No. 4,816,467, which is incorporated herein by reference), and include the following. For example, "vertebrate antibodies" refers to antibodies that are tetramers or aggregates thereof, comprising light and heavy chains which are usually aggregated in a "Y" configuration and which may or may not have covalent linkages between the chains. In vertebrate antibodies, the amino acid sequences of the chains are homologous with those sequences found in antibodies produced in vertebrates, whether in situ or in vitro (for example, in hybridomas). Vertebrate antibodies include, for example, purified polyclonal antibodies and monoclonal antibodies, methods for the preparation of which are described infra.

"Hybrid antibodies" are antibodies where chains are separately homologous with reference to mammalian antibody chains and represent novel assemblies of them, so that two different antigens are precipitable by the tetramer or aggregate. In hybrid antibodies, one pair of heavy and light chains are homologous to those found in an antibody raised against a first antigen, while a second pair of chains are homologous to those found in an antibody raised against a second antibody. This results in the property of "divalence", i.e., the ability to bind two antigens simultaneously. Such hybrids can also be formed using chimeric chains, as set forth below.

"Chimeric antibodies" refers to antibodies in which the heavy and/or light chains are fusion proteins. Typically, one portion of the amino acid sequences of the chain is homologous to corresponding sequences in an antibody derived from a particular species or a particular class, while the remaining segment of the chain is homologous to the sequences derived from another species and/or class. Usually, the variable region of both light and

heavy chains mimics the variable regions or antibodies derived from one species of vertebrates, while the constant portions are homologous to the sequences in the antibodies derived from another species of vertebrates. However, the definition is not limited to this particular example. Also included is any antibody in which either or both of the heavy or light chains are composed of combinations of sequences mimicking the sequences in antibodies of different sources, whether these sources be from differing classes or different species of origin, and whether or not the fusion point is at the variable/constant boundary. Thus, it is possible to produce antibodies in which neither the constant nor the variable region mimic known antibody sequences. It then becomes possible, for example, to construct antibodies whose variable region has a higher specific affinity for a particular antigen, or whose constant region can elicit enhanced complement fixation, or to make other improvements in properties possessed by a particular constant region.

Another example is "altered antibodies", which refers to antibodies in which the naturally occurring amino acid sequence in a vertebrate antibody has been varies. Utilizing recombinant DNA techniques, antibodies can be redesigned to obtain desired characteristics. The possible variations are many, and range from the changing of one or more amino acids to the complete redesign of a region, for example, the constant region. Changes in the constant region, in general, to attain desired cellular process characteristics, e.g., changes in complement fixation, interaction with membranes, and other effector functions. Changes in the variable region can be made to alter antigen-binding characteristics. The antibody can also be engineered to aid the specific delivery of a molecule or substance to a specific cell or tissue site. The desired alterations can be made by known techniques in molecular biology, e.g., recombinant techniques, site-directed mutagenesis, etc.

Yet another example are "univalent antibodies", which are aggregates comprised of a heavy-chain/light-chain dimer bound to the Fc (i.e., stem) region of a second heavy chain. This type of antibody escapes antigenic modulation. See, e.g., Glennie et al. Nature 295: 712 (1982). Included also within the definition of antibodies are "Fab" fragments of antibodies. The "Fab" region refers to those portions of the heavy and light chains which are roughly equivalent, or analogous, to the sequences which comprise the branch portion of the heavy and light chains, and which have been shown to exhibit immunological binding to a specified antigen, but which lack the effector Fc portion. "Fab" includes aggregates of one heavy and one light chain (commonly known as Fab'), as well as tetramers containing the 2H and 2L chains (referred to as F(ab)2), which are capable of selectively reacting with a designated antigen or antigen family. Fab antibodies can be divided into subsets analogous to those

described above, *i.e.*, "vertebrate Fab", "hybrid Fab", "chimeric Fab", and "altered Fab". Methods of producing Fab fragments of antibodies are known within the art and include, for example, proteolysis, and synthesis by recombinant techniques.

"Antigen-antibody complex" refers to the complex formed by an antibody that is specifically bound to an epitope on an antigen.

By "binds specifically" is meant high avidity and/or high affinity binding of an antibody to a specific polypeptide *i.e.*, epitope of a PrP^{SC} protein. Antibody binding to its epitope on this specific polypeptide is preferably stronger than binding of the same antibody to any other epitope, particularly those which may be present in molecules in association with, or in the same sample, as the specific polypeptide of interest *e.g.*, binds more strongly to PrP^{SC} than denatured fragments of PrP^{C} so that by adjusting binding conditions the antibody binds almost exclusively to PrP^{SC} and not denatured fragments of PrP^{C}. Antibodies that bind specifically to a polypeptide of interest may be capable of binding other polypeptides at a weak, yet detectable, level (*e.g.*, 10% or less of the binding shown to the polypeptide of interest). Such weak binding, or background binding, is readily discernible from the specific antibody binding to the compound or polypeptide of interest, *e.g.*, by use of appropriate controls. In general, antibodies of the invention bind pathogenic prions with a binding affinity of greater than 10^6 mole/l, more preferably greater than 10^7 mole/l or even more preferably 10^8mole/liters or more.

Techniques for determining amino acid sequence "similarity" or "percent identity" are well known in the art. In general, "similarity" means the amino acid to amino acid comparison of two or more polypeptides at the appropriate place, where amino acids are identical or possess similar chemical and/or physical properties such as charge or hydrophobicity. A so-termed "percent similarity" then can be determined between the compared polypeptide sequences. Techniques for determining nucleic acid and amino acid sequence identity also are well known in the art and include determining the nucleotide sequence of the mRNA for that gene (usually via a cDNA intermediate) and determining the amino acid sequence encoded thereby, and comparing this to a second amino acid sequence. In general, "identity" refers to an exact nucleotide to nucleotide or amino acid to amino acid correspondence of two polynucleotides or polypeptide sequences, respectively.

Two or more polynucleotide sequences can be compared by determining their "percent identity." Two or more amino acid sequences likewise can be compared by determining their "percent identity." The percent identity of two sequences, whether nucleic acid or peptide sequences, is generally described as the number of exact matches between

two aligned sequences divided by the length of the shorter sequence and multiplied by 100. An approximate alignment for nucleic acid sequences is provided by the local homology algorithm of Smith and Waterman, Advances in Applied Mathematics 2:482-489 (1981). This algorithm can be extended to use with peptide sequences using the scoring matrix developed by Dayhoff, Atlas of Protein Sequences and Structure, M.O. Dayhoff ed., 5 suppl. 3:353-358, National Biomedical Research Foundation, Washington, D.C., USA, and normalized by Gribskov, Nucl. Acids Res. 14(6):6745-6763 (1986). An implementation of this algorithm for nucleic acid and peptide sequences is provided by Accelrys in their BestFit application. The default parameters for this method are described by the supplier (Accelrys). Other equally suitable programs for calculating the percent identity or similarity between sequences are generally known in the art.

For example, percent identity of a particular nucleotide sequence to a reference sequence can be determined using the homology algorithm of Smith and Waterman with a default scoring table and a gap penalty of six nucleotide positions. Another method of establishing percent identity in the context of the present invention is to use the MPSRCH package of programs copyrighted by the University of Edinburgh, developed by John F. Collins and Shane S. Sturrok, and distributed by IntelliGenetics, Inc. (Mountain View, CA). From this suite of packages, the Smith-Waterman algorithm can be employed where default parameters are used for the scoring table (for example, gap open penalty of 12, gap extension penalty of one, and a gap of six). From the data generated, the "Match" value reflects "sequence identity." Other suitable programs for calculating the percent identity or similarity between sequences are generally known in the art, such as the alignment program BLAST, which can also be used with default parameters. For example, BLASTN and BLASTP can be used with the following default parameters: genetic code = standard; filter = none; strand = both; cutoff = 60; expect = 10; Matrix = BLOSUM62; Descriptions = 50 sequences; sort by = HIGH SCORE; Databases = non-redundant, GenBank + EMBL + DDBJ + PDB + GenBank CDS translations + Swiss protein + Spupdate + PIR. Details of these programs can be found at the following internet address: http://www.ncbi.nlm.gov/cgi-bin/BLAST. One of skill in the art can readily determine the proper search parameters to use for a given sequence in the above programs. For example, the search parameters may vary based on the size of the sequence in question.

Computer programs are also available to determine the likelihood of certain polypeptides forming structures such as β-helices. One such program, described herein, is the "ALB" program for protein and polypeptide secondary structure calculation and

predication. In addition, secondary protein structure can be predicted from the primary amino acid sequence, for example using protein crystal structure and aligning the protein sequence related to the crystal structure (*e.g.*, using Molecular Operating Environment (MOE) programs available from the Chemical Computing Group Inc., Montreal, P.Q., Canada). Other methods of predicting secondary structures are described, for example, in Garnier et al. (1996) *Methods Enzymol.* 266:540-553; Geourjon et al. (1995) *Comput. Applic. Biosci.* 11:681-684; Levin (1997) *Protein Eng.* 10:771-776; and Rost et al. (1993) *J. Molec. Biol.* 232:584-599.

Homology can also be determined by hybridization of polynucleotides under conditions that form stable duplexes between homologous regions, followed by digestion with single-stranded-specific nuclease(s), and size determination of the digested fragments. Two DNA, or two polypeptide sequences are "substantially homologous" to each other when the sequences exhibit at least about 80%-85%, preferably at least about 90%, and most preferably at least about 95%-98% sequence identity over a defined length of the molecules, as determined using the methods above. As used herein, substantially homologous also refers to sequences showing complete identity to the specified DNA or polypeptide sequence. DNA sequences that are substantially homologous can be identified in a Southern hybridization experiment under, for example, stringent conditions, as defined for that particular system. Defining appropriate hybridization conditions is within the skill of the art. See, e.g., Sambrook et al., *supra*; *DNA Cloning*, *supra*; *Nucleic Acid Hybridization*, *supra.*

An "immunogenic composition" as used herein refers to any composition (*e.g.*, polypeptides and/or polynucleotides) where administration of the composition to a subject results in the development in the subject of a humoral and/or a cellular immune response. The immunogenic composition can be introduced directly into a recipient subject, such as by injection, inhalation, oral, intranasal or any other parenteral or mucosal (*e.g.*, intra-rectally or intra-vaginally) route of administration.

By "epitope" is meant a site on an antigen to which specific B cells and/or T cells respond, rendering the molecule including such an epitope capable of eliciting an immunological reaction or capable of reacting with antibodies present in a biological sample. The term is also used interchangeably with "antigenic determinant" or "antigenic determinant site." An epitope can comprise 3 or more amino acids in a spatial conformation unique to the epitope. Generally, an epitope consists of at least 5 such amino acids and, more usually, consists of at least 8-10 such amino acids. Methods of determining spatial conformation of amino acids are known in the art and include, for example, x-ray crystallography and 2-

dimensional nuclear magnetic resonance. Furthermore, the identification of epitopes in a given protein is readily accomplished using techniques well known in the art, such as by the use of hydrophobicity studies and by site-directed serology. See, also, Geysen et al., *Proc. Natl. Acad. Sci. USA* (1984) 81:3998-4002 (general method of rapidly synthesizing peptides to determine the location of immunogenic epitopes in a given antigen); U.S. Patent No. 4,708,871 (procedures for identifying and chemically synthesizing epitopes of antigens); and Geysen et al., *Molecular Immunology* (1986) 23:709-715 (technique for identifying peptides with high affinity for a given antibody). Antibodies that recognize the same epitope can be identified in a simple immunoassay showing the ability of one antibody to block the binding of another antibody to a target antigen.

An "immunological response" or "immune response" as used herein is the development in the subject of a humoral and/or a cellular immune response to the prion chimera when the polypeptide is present in a vaccine composition. These antibodies may also neutralize infectivity, and/or mediate antibody-complement or antibody dependent cell cytotoxicity to provide protection to an immunized host. Immunological reactivity may be determined in standard immunoassays, such as a competition assays, well known in the art.

"Gene transfer" or "gene delivery" refers to methods or systems for reliably inserting DNA of interest into a host cell. Such methods can result in transient expression of non-integrated transferred DNA, extrachromosomal replication and expression of transferred replicons (e.g., episomes), or integration of transferred genetic material into the genomic DNA of host cells. Gene delivery expression vectors include, but are not limited to, vectors derived from alphaviruses, pox viruses and vaccinia viruses. When used for immunization, such gene delivery expression vectors may be referred to as vaccines or vaccine vectors.

The term "sample" includes biological samples derived from an animal (living or dead) such as organs (*e.g.,* brain, liver, kidney, etc), whole blood, blood fractions, plasma, cerebrospinal fluid (CSF), urine, tears, tissue, organs, biopsies and the like as well as pharmaceuticals, foods, cosmetics and the like.

The terms "label" and "detectable label" refer to a molecule capable of detection, including, but not limited to, radioactive isotopes, fluorescers, chemiluminescers, enzymes, enzyme substrates, enzyme cofactors, enzyme inhibitors, chromophores, dyes, metal ions, metal sols, ligands (e.g., biotin or haptens) and the like. The term "fluorescer" refers to a substance or a portion thereof that is capable of exhibiting fluorescence in the detectable range. Particular examples of labels that may be used with the invention include, but are not limited to fluorescein, rhodamine, dansyl, umbelliferone, Texas red, luminol, acradimum

esters, NADPH, beta-galactosidase, horseradish peroxidase, glucose oxidase, alkaline phosphatase and urease.

II. General Overview

Described herein are compositions comprising a prion chimera (and/or polynucleotides encoding prion chimeras), antibodies specific to pathogenic prions, and methods of making and using these prion chimeras and/or antibodies.

In general, the prion chimeras comprise a prion polypeptide and a non-prion polypeptide. Without being bound by one theory, it appears that the non-prion polypeptide serves as a backbone (or scaffold) for the prion polypeptide. The prion polypeptide is either incorporated into the chimera in a disease conformation or can be induced into a disease conformation after incorporation into the chimera (*e.g.*, by culture conditions and/or exposure to disease conformations). The chimeras allow for ready detection (diagnosis) of prion diseases and for the generation of antibodies (*e.g.*, therapeutic antibodies) against the disease conformation. In particular, the prion portion of the chimera may be involved in formation of a beta-helices that can be characteristic of disease-form proteins. By providing such chimeras, the disease-form protein(s) present in the sample can be ordered, aggregated or otherwise induced to a state that can then be detected. The antibodies and/or chimera (or one or more of their component parts) can be labeled or marked to facilitate detection.

III. Chimeras

Described herein are chimeras of at least one conformational disease protein (a protein or fragment thereof that assumes two different conformational shapes, one of which is associated with the disease) and a non-conformational disease protein (a protein or fragment thereof where changes in conformation are not associated with disease). Conformational diseases are exemplified herein by prion diseases.

III.A. Conformational Disease (Prion) Proteins

The following is a non-limiting list of diseases with associated insoluble proteins that assume two or more different conformations. In particularly preferred embodiments, the conformational disease protein is a prion and the chimera is a prion chimera.

Disease	Insoluble Protein(s)
Prion diseases (*e.g.*, Creutzfeld Jakob disease, scrapie, bovine spongiform encephalopathy)	PrP^{SC}
Alzheimer's Disease	APP, A* peptide, *1-antichymotrypsin, tan, non-A* component
ALS	SOD and neurofilament
Pick's disease	Pick body
Parkinson's disease	Lewy body
Diabetes Type 1	Amylin
Multiple myeloma – plasma cell dyscrasias	IgGL-chain
Familial amyloidotic polyneuropathy	Transthyretin
Medullary carcinoma of thyroid	Procalcitonin
Chronic Renal failure	beta2-microglobulin
Congestive heart failure	atrial natriuretic factor
senile cardiac and systemic amyloidosis	Transthyretin
Chronic inflammation	Serum amyloid A
Atherosclerosis Familial amyloidosis	ApoA1 Gelsolin

Further, the insoluble proteins listed above each include a number of variants or mutations that result in different strains that are all encompassed by the present invention. Known pathogenic mutations and polymorphisms in the PrP gene related to prion diseases are given below and the sequences of human, sheep and bovine are given in U.S. Pat. No. 5,565,186, issued Oct. 15, 1996.

MUTATION TABLE

Pathogenic Human mutations	Human polymorphisms	Sheep Polymorphisms	Bovine Polymorphisms
2 octarepeat insert 4 octarepeat insert	Codon 129 Met/Val Codon 219 Glu/Lys	Codon 171 Arg/Glu Codon 136 Ala/Val	5 or 6 octarepeats
5 octarepeat insert			
6 octarepeat insert 7 octarepeat insert			
8 octarepeat insert			
9 octarepeat insert			
Codon 102 Pro-Leu			
Codon 105 Pro-Leu			
Codon 117 Ala-Val			

Codon 145 Stop			
Codon 178 Asp-Asn			
Codon 180 Val-Ile			
Codon 198 Phe-Ser			
Codon 200 Glu-Lys			
Codon 210 Val-Ile			
Codon 217 Asn-Arg Codon 232 Met-Ala			

It should also be noted that prion proteins (and other conformational disease proteins) have two different 3-dimensional conformations with the same amino acid sequence. One conformation is associated with disease characteristics and is generally insoluble whereas the other conformation is not associated with disease characteristics and is soluble. Although exemplified with respect to prion proteins, the present invention is not limited to the diseases, proteins and strains listed.

As noted above, a conformational conversion of the non-pathogenic "cellular" forms into pathogenic isoforms is believed to be fundamental event in the onset of conformational diseases. During this pathogenic conversion, helix H1 and two adjacent surface loops L2 and L3 of the normal prion protein are thought to undergo a conformational transition into an extended beta-like structure, which is prompted by interactions with the pre-existing beta-sheet. Thus, in certain embodiments, the prion component used in the invention comprises an amino acid sequence of a prion protein that can adapt the β-helical conformation of a pathogenic prion protein. Discussion of the proposed β-helical structure of pathogenic prion proteins can be found in Wille, et al., "Structural Studies of the Scrapie Prion Protein by Electron Crystallography", *Proc. Natl. Acad. Sci. USA*, 99 (6): 3563-3568 (2002).

β-helices are known to contain highly ordered, stacked side chains on both the inside and outside of the helix. This side-chain stacking adds to the rigid geometry of the β-helices, which tend to have planar sides and essentially no interstrand twist. β-helices include both left- and right-handed structures. The number of residues per β-helical turn for left-handed helices averages about 18 residues per helical turn. The number of residues per β-helical turn for right-handed helices can vary substantially, but averages about 24 residues per helical turn. *See* Wille, et al., "Structural Studies of the Scrapie Prion Protein by Electron Crystallography", *Proc. Natl. Acad. Sci. USA*, 99 (6): 3563-3568 (2002).

Non-limiting examples of conformation (prion) proteins suitable for use in the chimeras described herein include peptides that interact with helix H1 and beta-strand S2

(residues 142-166, as numbered relative to human PrP (SEQ ID NO:1)). In addition to the beta-sheet forming region, polypeptides derived from or designed to interact with other regions of disease-form proteins can also be used, including hydrophobic regions, metal binding domains and the like. The amino acid sequence AGAAAAGA, comprising residues 112-119 of a hydrophobic region of murine PrP (SEQ ID NO:2) has been shown to be amyloidogenic and evolutionarily conserved. See, *e.g.,* Wegner et al. (2002) J Gen Virol 83(Pt 5):1237-45.

Prion polypeptides components of the chimeras described herein may also be designed to include metal binding domains, including, but not limited to, the highly conserved octarepeat sequence PHGGGWGQ or HGGGW spanning residues 60-91 of human PrP (SEQ ID NO:1). Jackson et al. Proc Natl Acad Sci U S A 2001 Jul 17;98(15):8531-5; *see, also,* Burns et al. (2002) Biochemistry 41(12):3991-4001.

In one embodiment, the prion polypeptide comprises amino acid residues from about amino acid 135 to about amino acid 155 of the prion amino acid sequence (*e.g.,* human (SEQ ID NO:6) or mouse (SEQ ID NO:7). In another embodiment, the prion polypeptide comprises amino acid residues from about amino acid 126 to about 154 of a prion protein (*e.g.,* human (SEQ ID NO:12) or mouse (SEQ ID NO:13). Preferably, the prion polypeptide of the prion chimera adapts a β-helix conformation within the prion chimera. Further, the prion polypeptide segment of the chimeras described herein can include one or more amino acid replacements, additions, and deletions thereof.

The conformational (prion) protein of the chimera can be anywhere from 5 to about 75 amino acids long (or any value therebetween) or even longer, preferably from about 20 to 50 amino acids (or any value therebetween), and even more preferably between about 20 and 30 amino acids (or any value therebetween) in length. The polypeptides can be identical to naturally-occurring beta-helical forming regions of these conformation proteins. Alternatively, one or more residues may be different from wild-type, for example introducing polar, non-polar residues or aromatic residues in certain positions to favor beta-helix formation and/or pi-stacking. *See, also,* Wang and Hecht (2002) *Proc Natl Acad Sci U S A* Mar 5;99(5):2760-5; Gazit (2002) *FASEB J.* 16(1):77-83.

The polynucleotide and amino acid sequence for prion proteins produced by many different species are known, including human, mouse, sheep and cattle. Variants to these sequences exist within each species, including variants that are more likely to adapt the β-helical conformation of pathogenic prions. The prion polypeptides (or fragments thereof) used in the invention can comprise fragments or derivatives of the amino acid sequences of

any species that produces prion proteins. Derivatives of such prion proteins, including amino acid replacements, deletions, additions and other mutations to these sequences can also be used in the prion sequences. Preferably, any amino acid replacements, additions, and deletions of the prion protein sequence will not affect the ability of the prion polypeptide to adapt a β-helical conformation (*e.g.*, within the prion chimera).

In one embodiment, conservative amino acid replacements are preferred. Conservative amino acid replacements are those that take place within a family of amino acids that are related in their side chains. Genetically encoded amino acids are generally divided into four families: (1) acidic = aspartate, glutamate; (2) basic = lysine, arginine, histidine; (3) non-polar = alanine, valine, leucine, isoleucine, proline, phenylalanine, methionine, tryptophan; and (4) uncharged polar = glycine, asparagine, glutamine, cysteine, serine, threonine, tyrosine. Phenylalanine, tryptophan, and tyrosine are sometimes classified jointly as aromatic amino acids. For example, it is reasonable predictable that an isolated replacement of a leucine with an isoleucine or valine, an aspartate with a glutamate, a threonine with a serine, or a similar conservative replacement of an amino acid with a structurally related amino acid will not have a major effect on the biological activity.

The prion proteins used in the invention may further comprise amino acids from portions of the prion that do not adapt a β-helical conformation. Preferably, these additional amino acids do not interfere with the ability of the prion polypeptide to adapt a β-helical conformation.

III.B. Non-Conformational Disease (Non-Prion) Proteins

The chimeras described herein will also comprise a sequence derived from a non-conformational disease, for example a fragment of a non-prion β-helical protein. Virtually any protein can serve as the non-prion portion of the chimera, although it is preferred that the non-prion portion of the chimera be capable of adapting a β-helical conformation, for example to facilitate and/or maintain that pathogenic conformation of the prion portion. Thus, preferably, the non-prion protein used in the chimera acts as a carrier (or scaffold or backbone) protein and allows the prion polypeptide (or fragment thereof) to adapt its β-helical conformation in the prion chimera.

Non-prion β-helical protein suitable for use in the invention include proteins with right handed β-helices, such as the *Bordetella pertussis* pertactin protein, and proteins with left-handed β-helices, such as *Methanosarcina thermophila* γ carbonic anhydrase. (See, *e.g.*,

Emsley et al. (1996) *Nature* May 2;381(6577):90-2 for a description of the structure of pertactin and Smith et al. (1999) *J Bacteriol* Oct;181(20):6247-53 for a description of carbonic anhydrase). Other right- or left-handed beta helical proteins suitable for use as described herein will be known to those of skill in the art, including, but not limited to, right handed beta-helix proteins such as pectate lyase, pectin lyase, galacturonase, chondroitinase B, pectin methylesterase, P22 tailspike protein, insect cysteine-rich antifreeze protein, cell-division inhibitor MinC, alpha subunit of glutamate synthase (*e.g.*, C-terminal domain) and C-terminal domain of adenylylcyclase associated protein as well as left handed beta-helix proteins such as UDP N-acetylglucosamine acyltransferase, galactoside acetyltransferase, xenobiotic acetyltransferase, tetrahydrodipicolinate-N-succinlytransferase, THDP-succinlytransferase, and DapD N-acetylglucosamine 1-phosphate uridyltransferase GlmU.

One or more amino acids of the non-prion protein of the chimera may be replaced with a prion polypeptide. Preferably, the prion polypeptide can adapt a β-helical conformation of a pathogenic prion. The prion portion may be located at either the C-terminal or the N-terminal region of the non-prion polypeptides. Where the prion chimera is located at the N-terminal region of the non-prion β-helical protein, preferably, the prion polypeptide is placed after the N-terminal methionine. Alternatively, the prion polypeptide may be inserted into (or embedded in) the non-prion portion. Non-limiting examples of prion chimeras are shown in FIGs. 3-6 and 8-23 (SEQ ID NOs:14-153).

The non-prion β-helical sequences of the prion chimera can further comprise fragments or derivatives of a non-prion β-helical proteins. For instance, amino acids can be removed from either the N terminal or the C terminal regions. The prion chimeras of the invention may further comprise additional segments. A non-limiting example of an additional segment is a tagging sequence. This tagging sequence can facilitate purification and solubilization of the chimera. Suitable tagging sequences are known in the art and include, for instance, sequences comprising histidine residues (*e.g.*, SEQ ID NO:5).

In certain embodiments, the non-prion polypeptide is derived from a *Bordetella pertussis* pertactin protein. Many variants of *Bordetella pertussis* pertactin protein are known in the art. (See, *e.g.*, FIG. 8 (SEQ ID NO:3)). P69 refers to the pertactin of a specific strain of pertussis. SEQ ID NO:3 represents the full-length sequence of the P69 protein, including all of the amino acids that are known to adapt the β-helical structure. P69 fragments suitable for use as the non-prion β-helical protein include the amino acid sequences selected from the group consisting of SEQ ID NO:8 and SEQ ID NO:9. SEQ ID NO:8 and 9 represent fragments of P69 where a portion of the amino acid sequence that adapts the β-helical

conformation has been removed. SEQ ID NO:8 is referred to in the figures as P69 Control A. SEQ ID NO:9 is referred to in the figures as P69 Control B. Accordingly, in one embodiment of the invention, the prion chimera comprises SEQ ID NO:8 and a prion protein (or fragment thereof). In another embodiment, the prion chimera comprises SEQ ID NO:9 and a prion portion. Non-limiting examples of prion chimeras using both human and mouse prion sequences and P69 non-prion β-helical protein sequences are shown in FIGs 3 – 6, 12 – 15, 20 – 23, 20 and 21.

Left-handed non-prion β-helical proteins can also be used in the invention. One example of a left handed non-prion β-helical protein suitable for use in the invention is *Methanosarcina thermophila* γ carbonic anhydrase (GCA). Many variants of GCA are known in the art. SEQ ID NO:4 represents the full-length sequence of GCA, including all of the amino acids that adapt the β-helical structure. In one embodiment, of the invention, a prion chimera comprising a polypeptide comprising SEQ ID NO:4 and a prion portion is used.

Examples of GCA non-prion β-helical protein fragments suitable for use in the invention include the amino acid sequences selected from the group consisting of SEQ ID NO:10 and SEQ ID NO:11. SEQ ID NOs:10 and 11 depicts fragments of GCA in which some of the amino acids that adapt the β-helical conformation have been removed. SEQ ID NO:10 is referred to in the figures as GCA Control A. SEQ ID NO. 11 is referred to in the figures as GCA Control B. Thus, in one embodiment of the invention, the prion chimera comprises a polypeptide comprising SEQ ID NO. 10 and a prion sequence. In another embodiment, the prion chimera comprises a polypeptide comprising SEQ ID NO. 11 and prion sequences. Non-limiting examples of prion chimeras using both human and mouse sequences and GCA non-prion β-helical proteins are shown in FIGs 8 – 11, 16 – 19, 22, 23 and 25-28.

Preferably, the prion chimeras used in the invention are not, themselves, pathogenic and/or infectious.

The prion chimeras used in the invention can be used to generate antibodies that specifically bind to prions in a pathogenic prion conformation. In addition, the prion chimeras themselves can be used to specifically bind to prions in a pathogenic prion conformation. Such specific binding generally refers to a greater affinity of the antibody or chimera to the pathogenic conformation relative to the nonpathogenic conformation, as can be determined by numerous suitable assays known in the art.

III.C. Polypeptide Production

The chimeras (and components thereof) of the present invention can be produced in any number of ways all of which are well known in the art.

In one embodiment, the polypeptides are generated using recombinant techniques, well known in the art. One of skill in the art could readily determining nucleotide sequences that encode the desired chimera using standard methodology and the teachings herein. Non-limiting examples of polynucleotide constructs encoding prion chimeras are depicted in SEQ ID NO:211-238 and the constructs depicted in Table 1.

Oligonucleotide probes can be devised based on the known sequences of the prion-encoding and non-prion-encoding polynucleotides and used to probe genomic or cDNA libraries. The sequences can then be further isolated using standard techniques and, *e.g.,* restriction enzymes employed to truncate the gene at desired portions of the full-length sequence. Similarly, sequences of interest can be isolated directly from cells and tissues containing the same, using known techniques, such as phenol extraction and the sequence further manipulated to produce the desired truncations. *See, e.g.,* Sambrook et al., *supra,* for a description of techniques used to obtain and isolate DNA.

The sequences encoding the chimera (or components thereof) can also be produced synthetically, for example, based on the known sequences. The nucleotide sequence can be designed with the appropriate codons for the particular amino acid sequence desired. The complete sequence is generally assembled from overlapping oligonucleotides prepared by standard methods and assembled into a complete coding sequence. *See, e.g.,* Edge (1981) *Nature* 292:756; Nambair *et al.* (1984) *Science* 223:1299; Jay *et al.* (1984) *J. Biol. Chem.* 259:6311; Stemmer et al. (1995) *Gene* 164:49-53.

Recombinant techniques are readily used to clone sequences encoding polypeptides useful in the claimed chimeras that can then be mutagenized *in vitro* by the replacement of the appropriate base pair(s) to result in the codon for the desired amino acid. Such a change can include as little as one base pair, effecting a change in a single amino acid, or can encompass several base pair changes. Alternatively, the mutations can be effected using a mismatched primer that hybridizes to the parent nucleotide sequence (generally cDNA corresponding to the RNA sequence), at a temperature below the melting temperature of the mismatched duplex. The primer can be made specific by keeping primer length and base composition within relatively narrow limits and by keeping the mutant base centrally located. See, *e.g.,* Innis et al, (1990) PCR Applications: Protocols for Functional Genomics; Zoller

and Smith, *Methods Enzymol.* (1983) 100:468. Primer extension is effected using DNA polymerase, the product cloned and clones containing the mutated DNA, derived by segregation of the primer extended strand, selected. Selection can be accomplished using the mutant primer as a hybridization probe. The technique is also applicable for generating multiple point mutations. See, e.g., Dalbie-McFarland et al. *Proc. Natl. Acad. Sci USA* (1982) 79:6409.

Once coding sequences for the desired components of chimera proteins have been isolated and/or synthesized, they can be cloned into any suitable vector or replicon for expression. (See, also, Examples). As will be apparent from the teachings herein, a wide variety of vectors encoding modified polypeptides can be generated by creating expression constructs which operably link, in various combinations, polynucleotides encoding prion and non-prion polypeptides having deletions or mutations therein.

Numerous cloning vectors are known to those of skill in the art, and the selection of an appropriate cloning vector is a matter of choice. Examples of recombinant DNA vectors for cloning and host cells which they can transform include the bacteriophage λ (*E. coli*), pBR322 (*E. coli*), pACYC177 (*E. coli*), pKT230 (gram-negative bacteria), pGV1106 (gram-negative bacteria), pLAFR1 (gram-negative bacteria), pME290 (non-*E. coli* gram-negative bacteria), pHV14 (*E. coli* and *Bacillus subtilis*), pBD9 (*Bacillus*), pIJ61 (*Streptomyces*), pUC6 (*Streptomyces*), YIp5 (*Saccharomyces*), YCp19 (*Saccharomyces*) and bovine papilloma virus (mammalian cells). *See, generally, DNA Cloning*: Vols. I & II, *supra*; Sambrook *et al.*, *supra*; B. Perbal, *supra.*

Insect cell expression systems, such as baculovirus systems, can also be used and are known to those of skill in the art and described in, *e.g.*, Summers and Smith, *Texas Agricultural Experiment Station Bulletin No. 1555* (1987). Materials and methods for baculovirus/insect cell expression systems are commercially available in kit form from, *inter alia*, Invitrogen, San Diego CA ("MaxBac" kit).

Plant expression systems can also be used to produce the prion chimeras. Generally, such systems use virus-based vectors to transfect plant cells with heterologous genes. For a description of such systems see, *e.g.,* Porta et al., *Mol. Biotech.* (1996) 5:209-221; and Hackland et al., *Arch. Virol.* (1994) 139:1-22.

Viral systems, such as a vaccinia based infection/transfection system, as described in Tomei et al., *J. Virol.* (1993) 67:4017-4026 and Selby et al., *J. Gen. Virol.* (1993) 74:1103-1113, will also find use with the present invention. In this system, cells are first transfected *in vitro* with a vaccinia virus recombinant that encodes the bacteriophage T7

RNA polymerase. This polymerase displays exquisite specificity in that it only transcribes templates bearing T7 promoters. Following infection, cells are transfected with the DNA of interest, driven by a T7 promoter. The polymerase expressed in the cytoplasm from the vaccinia virus recombinant transcribes the transfected DNA into RNA that is then translated into protein by the host translational machinery. The method provides for high level, transient, cytoplasmic production of large quantities of RNA and its translation product(s).

The gene can be placed under the control of a promoter, ribosome binding site (for bacterial expression) and, optionally, an operator (collectively referred to herein as "control" elements), so that the DNA sequence encoding the desired polypeptide is transcribed into RNA in the host cell transformed by a vector containing this expression construction. The coding sequence may or may not contain a signal peptide or leader sequence. With the present invention, both the naturally occurring signal peptides or heterologous sequences can be used. Leader sequences can be removed by the host in post-translational processing. *See, e.g.*, U.S. Patent Nos. 4,431,739; 4,425,437; 4,338,397. Such sequences include, but are not limited to, the TPA leader, as well as the honey bee mellitin signal sequence.

Other regulatory sequences may also be desirable which allow for regulation of expression of the protein sequences relative to the growth of the host cell. Such regulatory sequences are known to those of skill in the art, and examples include those which cause the expression of a gene to be turned on or off in response to a chemical or physical stimulus, including the presence of a regulatory compound. Other types of regulatory elements may also be present in the vector, for example, enhancer sequences.

The control sequences and other regulatory sequences may be ligated to the coding sequence prior to insertion into a vector. Alternatively, the coding sequence can be cloned directly into an expression vector that already contains the control sequences and an appropriate restriction site.

In some cases it may be necessary to modify the coding sequence so that it may be attached to the control sequences with the appropriate orientation; *i.e.*, to maintain the proper reading frame. Mutants or analogs may be prepared by the deletion of a portion of the sequence encoding the protein, by insertion of a sequence, and/or by substitution of one or more nucleotides within the sequence. Techniques for modifying nucleotide sequences, such as site-directed mutagenesis, are well known to those skilled in the art. *See, e.g.*, Sambrook *et al., supra*; *DNA Cloning*, Vols. I and II, *supra*; *Nucleic Acid Hybridization*, *supra*.

The expression vector is then used to transform an appropriate host cell. A number of mammalian cell lines are known in the art and include immortalized cell lines available from

the American Type Culture Collection (ATCC), such as, but not limited to, Chinese hamster ovary (CHO) cells, HeLa cells, baby hamster kidney (BHK) cells, monkey kidney cells (COS), human hepatocellular carcinoma cells (*e.g.*, Hep G2), Vero293 cells, as well as others. Similarly, bacterial hosts such as *E. coli*, *Bacillus subtilis*, and *Streptococcus spp.*, will find use with the present expression constructs. Yeast hosts useful in the present invention include *inter alia*, *Saccharomyces cerevisiae*, *Candida albicans*, *Candida maltosa*, *Hansenula polymorpha*, *Kluyveromyces fragilis*, *Kluyveromyces lactis*, *Pichia guillerimondii*, *Pichia pastoris*, *Schizosaccharomyces pombe* and *Yarrowia lipolytica*. Insect cells for use with baculovirus expression vectors include, *inter alia*, *Aedes aegypti*, *Autographa californica*, *Bombyx mori*, *Drosophila melanogaster*, *Spodoptera frugiperda*, and *Trichoplusia ni*.

Depending on the expression system and host selected, the proteins of the present invention are produced by growing host cells transformed by an expression vector described above under conditions whereby the protein of interest is expressed. The selection of the appropriate growth conditions is within the skill of the art.

In one embodiment, the transformed cells secrete the polypeptide product into the surrounding media. Certain regulatory sequences can be included in the vector to enhance secretion of the protein product, for example using a tissue plasminogen activator (TPA) leader sequence, an interferon (γ or α) signal sequence or other signal peptide sequences from known secretory proteins. The secreted polypeptide product can then be isolated by various techniques described herein, for example, using standard purification techniques such as but not limited to, hydroxyapatite resins, column chromatography, ion-exchange chromatography, size-exclusion chromatography, electrophoresis, HPLC, immunoadsorbent techniques, affinity chromatography, immunoprecipitation, and the like.

Alternatively, the transformed cells are disrupted, using chemical, physical or mechanical means, which lyse the cells yet keep the recombinant polypeptides substantially intact. Intracellular proteins can also be obtained by removing components from the cell wall or membrane, *e.g.*, by the use of detergents or organic solvents, such that leakage of the polypeptides occurs. Such methods are known to those of skill in the art and are described in, e.g., *Protein Purification Applications: A Practical Approach*, (E.L.V. Harris and S. Angal, Eds., 1990).

For example, methods of disrupting cells for use with the present invention include but are not limited to: sonication or ultrasonication; agitation; liquid or solid extrusion; heat treatment; freeze-thaw; desiccation; explosive decompression; osmotic shock; treatment with

lytic enzymes including proteases such as trypsin, neuraminidase and lysozyme; alkali treatment; and the use of detergents and solvents such as bile salts, sodium dodecylsulphate, Triton, NP40 and CHAPS. The particular technique used to disrupt the cells is largely a matter of choice and will depend on the cell type in which the polypeptide is expressed, culture conditions and any pre-treatment used.

Following disruption of the cells, cellular debris is removed, generally by centrifugation, and the intracellularly produced polypeptides are further purified, using standard purification techniques such as but not limited to, column chromatography, ion-exchange chromatography, size-exclusion chromatography, electrophoresis, HPLC, immunoadsorbent techniques, affinity chromatography, immunoprecipitation, and the like.

For example, one method for obtaining the intracellular polypeptides of the present invention involves affinity purification, such as by immunoaffinity chromatography using antibodies (*e.g.*, previously generated prion chimera specific antibodies), or by lectin affinity chromatography. Particularly preferred lectin resins are those that recognize mannose moieties such as but not limited to resins derived from *Galanthus nivalis* agglutinin (GNA), *Lens culinaris* agglutinin (LCA or lentil lectin), *Pisum sativum* agglutinin (PSA or pea lectin), *Narcissus pseudonarcissus* agglutinin (NPA) and *Allium ursinum* agglutinin (AUA). The choice of a suitable affinity resin is within the skill in the art. After affinity purification, the polypeptides can be further purified using conventional techniques well known in the art, such as by any of the techniques described above.

Polypeptides can be conveniently synthesized chemically, for example by any of several techniques that are known to those skilled in the peptide art. In general, these methods employ the sequential addition of one or more amino acids to a growing peptide chain. Normally, either the amino or carboxyl group of the first amino acid is protected by a suitable protecting group. The protected or derivatized amino acid can then be either attached to an inert solid support or utilized in solution by adding the next amino acid in the sequence having the complementary (amino or carboxyl) group suitably protected, under conditions that allow for the formation of an amide linkage. The protecting group is then removed from the newly added amino acid residue and the next amino acid (suitably protected) is then added, and so forth. After the desired amino acids have been linked in the proper sequence, any remaining protecting groups (and any solid support, if solid phase synthesis techniques are used) are removed sequentially or concurrently, to render the final polypeptide. By simple modification of this general procedure, it is possible to add more than one amino acid at a time to a growing chain, for example, by coupling (under conditions

which do not racemize chiral centers) a protected tripeptide with a properly protected dipeptide to form, after deprotection, a pentapeptide. See, e.g., J. M. Stewart and J. D. Young, Solid Phase Peptide Synthesis (Pierce Chemical Co., Rockford, IL 1984) and G. Barany and R. B. Merrifield, The Peptides: Analysis, Synthesis, Biology, editors E. Gross and J. Meienhofer, Vol. 2, (Academic Press, New York, 1980), pp. 3-254, for solid phase peptide synthesis techniques; and M. Bodansky, Principles of Peptide Synthesis, (Springer-Verlag, Berlin 1984) and E. Gross and J. Meienhofer, Eds., The Peptides: Analysis, Synthesis, Biology, Vol. 1, for classical solution synthesis. These methods are typically used for relatively small polypeptides, i.e., up to about 50-100 amino acids in length, but are also applicable to larger polypeptides.

Typical protecting groups include t-butyloxycarbonyl (Boc), 9-fluorenylmethoxycarbonyl (Fmoc) benzyloxycarbonyl (Cbz); p-toluenesulfonyl (Tx); 2,4-dinitrophenyl; benzyl (Bzl); biphenylisopropyloxycarboxy-carbonyl, t-amyloxycarbonyl, isobornyloxycarbonyl, o-bromobenzyloxycarbonyl, cyclohexyl, isopropyl, acetyl, o-nitrophenylsulfonyl and the like.

Typical solid supports are cross-linked polymeric supports. These can include divinylbenzene cross-linked-styrene-based polymers, for example, divinylbenzene-hydroxymethylstyrene copolymers, divinylbenzene-chloromethylstyrene copolymers and divinylbenzene-benzhydrylaminopolystyrene copolymers.

The polypeptide analogs of the present invention can also be chemically prepared by other methods such as by the method of simultaneous multiple peptide synthesis. See, e.g., Houghten *Proc. Natl. Acad. Sci. USA* (1985) 82:5131-5135; U.S. Patent No. 4,631,211.

IV. Antibodies

The antibodies of the invention are preferably specific and selective for pathogenic prions and can be used to distinguish between pathogenic and non-pathogenic prions. Preferably, the antibodies of the invention recognize a β-helical region of a pathogenic prion. In one embodiment, the antibodies of the invention are generated by administering a prion chimera or polynucleotide encoding a prion chimera to an animal. The methods may also include isolating the antibodies from the animal.

The antibodies of the invention may be polyclonal or monoclonal antibody preparations, monospecific antisera, human antibodies, or may be hybrid or chimeric antibodies, such as humanized antibodies, altered antibodies $(Fab')_2$ fragments, F(ab)

fragments, Fv fragments, single-domain antibodies, dimeric or trimeric antibody fragments or constructs, minibodies, or functional fragments thereof which bind to the antigen in question.

Antibodies are produced using techniques well known to those of skill in the art and disclosed in, for example, U.S. Patent Nos. 4,011,308; 4,722,890; 4,016,043; 3,876,504; 3,770,380; and 4,372,745. For example, polyclonal antibodies are generated by immunizing a suitable animal, such as a mouse, rat, rabbit, sheep, or goat, with an antigen of interest (*e.g.*, prion chimera). In order to enhance immunogenicity, the antigen can be linked to a carrier prior to immunization. Such carriers are well known to those of ordinary skill in the art. Immunization is generally performed by mixing or emulsifying the antigen in saline, preferably in an adjuvant such as Freund's complete adjuvant, and injecting the mixture or emulsion parenterally (generally subcutaneously or intramuscularly). The animal is generally boosted 2 – 6 weeks later with one or more injections of the antigen in saline, preferably using Freund's incomplete adjuvant. Antibodies may also be generated by *in vitro* immunization, using methods known in the art. Polyclonal antiserum is then obtained from the immunized animal.

Monoclonal antibodies are generally prepared using the method of Kohler and Milstein (1975) *Nature* 256:495-497, or a modification thereof. Typically, a mouse or rat is immunized as described above. However, rather than bleeding the animal to extract serum, the spleen (and optionally several large lymph nodes) is removed and dissociated into single cells. If desired, the spleen cells may be screened (after removal of nonspecifically adherent cells) by applying a cell suspension to a plate or well coated with the antigen. B-cells, expressing membrane-bound immunoglobulin specific for the antigen, will bind to the plate, and are not rinsed away with the rest of the suspension. Resulting B-cells, or all dissociated spleen cells, are then induced to fuse with myeloma cells for form hybridomas, and are cultured in a selective medium (*e.g.*, hypoxanthine, aminopterin, thymidine medium, "HAT"). The resulting hybridomas are plated by limiting dilution, and are assayed for the production of antibodies that bind specifically to the immunizing antigen (and which do not bind to unrelated antigens). The selected monoclonal antibody-secreting hybridomas are then cultured either in vitro (*e.g.*, in tissue culture bottles or hollow fiber reactors), or *in vivo* (*e.g.*, as ascites in mice).

Humanized and chimeric antibodies are also useful in the invention. Hybrid (chimeric) antibody molecules are generally discussed in Winter et al. (1991) *Nature* 349: 293-299 and U.S. Patent No. 4,816,567. Humanized antibody molecules are generally discussed in Riechmann et al. (1988) *Nature* 332:323-327; Verhoeyan et al. (1988) *Science*

239:1534-1536; and U.K. Patent Publication No. GB 2,276,169, published 21 September 1994). One approach to engineering a humanized antibody involves cloning recombinant DNA containing the promoter, leader, and variable-region sequences from a mouse antibody gene and the constant-region exons from a human antibody gene to create a mouse-human chimera, a humanized antibody. See generally, Kuby, "Immunology, 3rd Edition", W.H. Freeman and Company, New York (1998) at page 136.

Antibodies, both monoclonal and polyclonal, which are directed against chimeras as described herein are particularly useful in diagnosis and therapeutic applications, for example, those antibodies that are neutralizing are useful in passive immunotherapy. Monoclonal antibodies, in particular, may be used to raise anti-idiotype antibodies.

Anti-idiotype antibodies are immunoglobulins that carry an "internal image" of the antigen of the agent against which protection is desired. Techniques for raising anti-idiotype antibodies are known in the art. See, e.g., Grzych (1985), Nature 316:74; MacNamara et al. (1984), Science 226:1325, Uytdehaag et al (1985), J. Immunol. 134:1225. These anti-idiotype antibodies may also be useful for treatment and/or diagnosis of conformational diseases.

Antibody fragments that retain the ability to recognize the pathogenic prion conformation are also included within the scope of the invention. A number of antibody fragments are known in the art that comprise antigen-binding sites capable of exhibiting immunological binding properties of an intact antibody molecule. For example, functional antibody fragments can be produced by cleaving a constant region, not responsible for antigen binding, from the antibody molecule, using e.g., pepsin, to produce $F(ab')_2$ fragments. These fragments will contain two antigen binding sites, but lack a portion of the constant region from each of the heavy chains. Similarly, if desired, Fab fragments, comprising a single antigen binding site, can be produced, e.g., by digestion of polyclonal or monoclonal antibodies with papain. Functional fragments, including only the variable regions of the heavy and light chains, can also be produced, using standard techniques such as recombinant production or preferential proteolytic cleavage of immunoglobulin molecules. These fragments are known as F_v. See, *e.g.*, Inbar et al. (1972) *Proc. Nat. Acad. Sci USA* 69:2659-2662; Hochman et al. (1976) *Biochem* 15:2706-2710; and Ehrlich et al. (1980) *Biochem* 19:4091-4096.

A single-chain Fv ("sFv" or scFv") polypeptide is a covalently linked $V_H - V_L$ heterodimer that is expressed from a gene fusion including V_H – and V_L- encoding genes linked by a peptide-encoding linker. Huston et al. (1988) *Proc. Nat. Acad. Sci. USA* 85:5879-5883. A number of methods have been described to discern and develop chemical structures

(linkers) for converting the naturally aggregated, but chemically separated, light and heavy polypeptide chains from an antibody V region into an sFv molecule which will fold into a three dimensional structure substantially similar to the structure of an antigen-binding site. See, e.g., U.S. Patent Nos. 5,091,513; 5,132,405; and 4,946,778. The sFv molecules may be produced using methods described in the art. See, e.g., Huston et al. (1988) *Proc. Nat. Acad. Sci USA* 85:5879-5338; U.S. Patent Nos. 5,091,513; 5,132,405 and 4,946,778. Design criteria include determining the appropriate length to span the distance between the C-terminus of one chain and the N-terminus of the other, wherein the linker is generally formed from small hydrophilic amino acid residues that do not coil or form secondary structures. Such methods have been described in the art. See, e.g., U.S. Patent Nos. 5,091,513; 5,132,405 and 4,946,778. Suitable linkers generally comprise polypeptide chains of alternating sets of glycine and serine residues, and may include glutamic acid and lysine residues inserted to enhance solubility.

"Mini-antibodies" or "minibodies" will also find use with the present invention. Minibodies are sFv polypeptide chains that include oligomerization domains at their C-termini, separated from the sFv by a hinge region. Pack et al., (1992) *Biochem* 31:1579-1584. The oligomerization domain comprises self-associating α-helices, e.g., leucine zippers, that can be further stabilized by additional disulfide bonds. The oligomerization domain is designed to be compatible with vectorial folding across a membrane, a process thought to facilitate in vivo folding of the polypeptide into a functional binding protein. Generally, minibodies are produced using recombinant methods well known in the art. See, e.g., Pack et al., (1992) *Biochem* 31:1579-1584; Cumber et al. (1992) *J. Immunology* 149B:120-126.

Non-conventional means can also be used to generate and identify the antibodies of the invention. For example, a phage display library can be screened for antibodies that bind to the prion chimeras of the invention. Preferably, the antibody will be specific to the β-helical conformation of a pathogenic prion. See generally, Siegel, "Recombinant Monoclonal Antibody Technology", *Transfus. Clin. Biol.* (2002) 9(1): 15-22; Sidhu, "Phage Display in Pharmaceutical Biotechnology", *Curr. Opin. Biotechnol.* (2000) 11(6):610 – 616; Sharon, et al., "Recombinant Polyclonal Antibody Libraries", *Comb. Chem. High Throughput Screen* (2000) 3(3): 185-196; and Schmitz et al., "Phage Display: A Molecular Tool for the Generation of Antibodies – Review", *Placenta*, (2000) 21 SupplA: S106-12.

As noted above, the antibodies of the invention may also be generated by administering a polynucleotide sequence encoding a prion chimera into an animal. When the prion chimera is expressed *in vivo*, antibodies specific to the prion chimera are generated *in*

vivo. Methods for polynucleotide delivery of the prion chimeras of the invention are discussed in section VI.A., below.

The specificity of the antibodies of the invention can be tested in a variety of ways. For example, as mentioned above, prions having a pathogenic conformation are generally resistant to certain proteases, such as proteinase K. The same proteases are able to degrade prions in a nonpathogenic conformation. One method of testing the specificity of the antibodies of the present invention is to select a biological sample containing both pathogenic and non-pathogenic prions. The sample can be separated into two equal volumes. Antibodies of the invention can be added adsorbed onto a solid support (as further described below) and used to obtain a quantitative value directly related to the number of antibody-prion binding interactions on the solid support. Protease can be added to the second sample and the same test performed. Because the protease in the second sample will degrade any non-pathogenic prions, any antibody-prion binding interactions in the second volume can be attributed to pathogenic prions. Variations and other assays known in the art can also be used to demonstrate the specificity of the antibodies of the invention.

V. Assays

The prion chimeras, polynucleotides and/or antibodies of the invention can be used in a variety of assays to screen samples (*e.g.*, biological samples) to detect the presence of pathogenic prions.

Assays involving antibodies may use, for example, a monoclonal antibody directed towards a single epitope, a combination of monoclonal antibodies directed towards the same epitope(s), monoclonal antibodies directed towards different epitopes, monoclonal antibodies directed to epitopes of different chimeras, polyclonal antibodies directed towards the same antigen, polyclonal antibodies directed towards different antigens and/or a combination of monoclonal and polyclonal antibodies.

For example, the antibodies of the invention can be used to capture a pathogenic prion in a biological sample. Any suitable means of detection can then be used to identify binding between the antibody and a pathogenic prion. Thus, in one embodiment, the invention includes a method of detecting pathogenic forms of prion particles in a sample (*e.g.*, a biological sample), the method comprising (a) exposing the sample suspected of containing a pathogenic prion to a first antibody, wherein the first antibody is specific to pathogenic prions; and (b) detecting the presence or absence of the first antibody binding to a pathogenic

prion. Preferably, the antibody is generated according to any of the methods described herein.

The prion chimeras of the invention themselves can also be used to capture a pathogenic prion in a sample. Means of detection can then be used to identify binding between the prion chimera and a pathogenic prion. Thus, in certain embodiments, the invention includes a method of detecting a pathogenic prion in a biological sample, the method comprising (a) exposing the biological sample suspected of containing a pathogenic prion to a prion chimera; and (b) detecting the presence or absence of the prion chimera bound to the pathogenic prion.

In addition, assay (*e.g.*, immunoassay) protocols may be based, for example, upon competition, or direct reaction, or sandwich type assays. Protocols may also, for example, use solid supports, or may be by immunoprecipitation. Most assays involve the use of labeled antibody or polypeptide (*e.g.*, chimera). The labels may be, for example, fluorescent, chemiluminescent, radioactive, or dye molecules. Detectable labels suitable for use in the invention include any molecule capable of detection, including, but not limited to, radioactive isotopes, fluorescers, chemiluminescers, chromophores, enzymes, enzyme substrates, enzyme cofactors, enzyme inhibitors, chromophores, dyes, metal ions, metal sols, ligands (e.g., biotin, strepavidin or haptens) and the like. Additional labels include, but are not limited to, those which use fluoresce, including those substances or portions thereof which are capable of exhibiting fluorescence in the detectable range. Particular examples of labels that may be used in the invention include, but are not limited to, horse radish peroxidase (HRP), fluorescein, FITC, rhodamine, dansyl, umbelliferone, dimethyl acridinium ester (DMAE), Texas red, luminol, NADPH and β-galactosidase.

Assays that amplify the signals from the probe are also known. Examples of which are assays that utilize biotin and avidin, and enzyme-labeled and mediated immunoassays, such as ELISA assays.

Any of the assays described herein may be conduced in solution (*e.g.*, a liquid medium) or a solid medium. In one embodiment, the antibodies and/or prion chimeras are exposed to the biological sample in a liquid medium. The presence or absence of binding between the antibody and/or prion chimera and a pathogenic prion in the biological sample can be detected in several ways. In one method, immunoprecipitation is used to separate out antibody and/or prion chimeras that are bound to the pathogenic prion. Preferably, the immunoprecipitation is facilitated by the addition of a precipitating enhancing agent. A precipitation enhancing agent includes moieties that can enhance or increase the precipitation

of the antibodies and/or prion chimeras that are bound to pathogenic prions. Such precipitation enhancing agents include polyethylene glycol (PEG), protein G, protein A and the like. Where protein G or protein A are used as precipitation enhancing agents, the protein can optionally be attached to a bead, preferably a magnetic bead. Precipitation can be further enhanced by use of centrifugation or with the use of magnetic force. Use of such precipitating enhancing agents are known in the art.

In certain embodiments, the antibodies and the chimeras of the invention can be adsorbed onto solid supports for use in the assays of the invention. A solid support, for purposes of the invention, can be any material that is an insoluble matrix and can have a rigid or semi-rigid surface. Exemplary solid supports include, but are not limited to, substrates such as nitrocellulose (e.g., in membrane or microtiter well form); polyvinylchloride (e.g., sheets or microtiter wells); polystyrene latex (e.g., beads or microtiter plates); polyvinyl fluoride; diazotized paper; nylon membranes; activated beads, magnetically responsive beads, and the like. Particular supports include plates, pellets, disks, capillaries, hollow fibers, needles, pins, solid fibers, cellulose beads, pore-glass beads, silica gels, polystyrene beads optionally cross-linked with divinylbenzene, grafted co-poly beads, polyacrylamide beads, latex beads, dimethylacrylamide beads optionally crosslinked with N-N'-bis-acryloylethylenediamine, and glass particles coated with a hydrophobic polymer.

If desired, the molecules to be added to the solid support can readily be functionalized to create styrene or acrylate moieties, thus enabling the incorporation of the molecules into polystyrene, polyacrylate or other polymers such as polyimide, polyacrylamide, polyethylene, polyvinyl, polydiacetylene, polyphenylene-vinylene, polypeptide, polysaccharide, polysulfone, polypyrrole, polyimidazole, polythiophene, polyether, epoxies, silica glass, silica gel, siloxane, polyphosphate, hydrogel, agarose, cellulose and the like.

Typically, the solid support coupled to an antibody or chimera is reacted with a sample (*e.g.,* biological sample). After washing, a secondary binder moiety (*e.g.,* antibodies specific to pathogenic prions or chimeras) is added under suitable binding conditions, such that the secondary binder is capable of associating selectively with the bound pathogenic prions, if any, in the sample. The presence of the secondary binder can then be detected using techniques well known in the art. A number of anti-human immunoglobulin (Ig) molecules are known in the art (e.g., commercially available goat anti-human Ig or rabbit anti-human Ig). Ig molecules for use herein will preferably be of the IgG or IgA type, however, IgM may also be appropriate in some instances. The Ig molecules can be readily conjugated to a detectable enzyme label, such as horseradish peroxidase, glucose oxidase,

Beta-galactosidase, alkaline phosphatase and urease, among others, using methods known to those of skill in the art. An appropriate enzyme substrate is then used to generate a detectable signal.

An exemplary sandwich type assay can be conducted using the antibodies and/or prion chimeras described herein as follows. A sample (*e.g.*, biological sample) suspected of containing a pathogenic prion is exposed to a first antibody specific to pathogenic prions (*e.g.*, an antibody generated using a prion chimera) and/or a prion chimera. Following a washing step, the presence or absence of the first antibody and/or the prion chimera bound to the pathogenic prion is determined. The presence or absence of binding between the first antibody and/or the prion chimera and a pathogenic prion can be facilitated by use of a detectable label. Such a detectable label could be placed on the first antibody and/or the prion chimera.

Alternatively, a "two antibody/chimera sandwich" assay can be used to detect pathogenic prions. In this technique, the solid support is reacted first with one or more of the antibodies directed against prion chimeras or prion chimeras themselves, washed and then exposed to the test sample. Antibodies or prion chimeras are again added and the reaction visualized using either a direct color reaction or using a labeled second antibody, such as an anti-immunoglobulin labeled with horseradish peroxidase, alkaline phosphatase or urease.

Typically, a solid support is first reacted with an antibody and/or chimera such that the antibody and/or chimera are sufficiently immobilized to the support. Sometimes, immobilization to the support can be enhanced by first coupling the antibody and/or chimera to a protein with better solid phase-binding properties. Suitable coupling proteins include, but are not limited to, macromolecules such as serum albumins including bovine serum albumin (BSA), keyhole limpet hemocyanin, immunoglobulin molecules, thyroglobuline, ovalbumin, and other proteins well known to those skilled in the art. Other reagents that can be used to bind molecules to the support include polysaccharides, polylactic acids, polyglycolic acids, polymeric amino acids, amino acid copolymers, and the like. Such molecules and methods of coupling these molecules to antigens, are well known to those of ordinary skill in the art. See, *e.g.*, Brinkley, M.A., (1992) *Bioconjugate Chem.*, 3:2-13; Hashida et al. (1984) *J. Appl. Biochem.*, 6:56-63; and Anjaneyulu and Staros (1987) *International J. of Peptide and Protein Res.* 30:117-124.

As noted above, after reacting the solid support with the antibody, chimera or other solid-phase components described above, any non-immobilized components may be removed from the support by washing, and the support-bound components are then contacted with a

biological sample suspected of containing pathogenic prions under suitable binding conditions. After washing to remove any unbound prions, a second antibody and/or a prion chimera may be added under suitable binding conditions. The added second antibody and/or prion chimera can include a detectable label.

Suitable controls can also be used in the assays of the invention. For instance, a negative control of PrP^C can be used in the assays. A positive control of a prion chimera could also be used in the assays. Such controls can optionally be detectably labeled.

Several variations and combinations using the antibodies and chimeras of the invention may be applied in the assays of the invention. The following non-limiting examples are described for illustration.

In a first example, an antibody specific to pathogenic prions (or a prion chimera) is adsorbed onto (immobilized on) a solid support, for example an antibody generated using a prion chimera. The solid support is combined with a sample (*e.g.*, a biological sample) suspected of containing a pathogenic prion, under conditions that allow a pathogenic prion, when present in the sample, to bind to the first antibody or to the prion chimera. The solid support can then be washed to remove any unbound materials. Then, a detectably labeled second antibody and/or a detectably labeled prion chimera can be added to the solid support under complex forming conditions. The second antibody (or labeled prion chimera) can be specific to pathogenic prions or it may be capable of binding to both pathogenic and non-pathogenic prions. Complexes formed between the first antibody and/or the prion chimera, a pathogenic prion from the biological sample, and the second detectably labeled antibody and/or the detectably labeled prion chimera may then be detected to determine the presence of pathogenic prions in the sample.

In another example, the invention includes a method of detecting the presence of a pathogenic prion particle in a sample (*e.g.*, a biological sample), the method comprising (a) providing a solid support comprising one or more of a first antibody specific to pathogenic prions (*e.g.*, an antibody generated according to the methods described herein) or a prion chimera bound thereto; (b) combining a sample with the solid support under conditions which allow a pathogenic prion, when present in a biological sample, to bind to the first antibody or to the prion chimera; (c) adding to the solid support, under complex forming conditions, one or more of a detectably labeled second antibody or a detectably labeled prion chimera, wherein the second antibody may optionally recognize both pathogenic and nonpathogenic prions; and (d) detecting complexes formed between the first antibody and/or prion chimera,

a pathogenic prion from the biological sample, and the second detectably labeled antibody and/or the detectably labeled prion chimera.

ELISA methods can be used, wherein the wells of a microtiter plate are coated with the first antibody and/or a prion chimera. A biological sample containing or suspected of containing pathogenic prions is then added to the coated wells. After a period of incubation sufficient to allow any pathogenic prions to bind to the first antibody and/or the prion chimeras, the plate(s) can be washed to remove unbound moieties and a detectably labeled secondary binding molecule, such as a second antibody or a prion chimera, added. This secondary binding molecular is allowed to react with any captured sample prion, the plate washed and the presence of the labeled antibodies and/or labeled chimeras detected using methods well known in the art.

In another embodiment, an antibody that can recognize both pathogenic and nonpathogenic prions and/or a prion chimera can be bound to the solid support. The solid support is then combined with a sample (*e.g.*, a biological sample) suspected of containing a pathogenic prion, under conditions which allow any prion proteins, both pathogenic and nonpathogenic, to bind to the first antibody and/or prion chimera. The solid support can then be washed to remove any unbound materials. Then, a detectably labeled second antibody and/or a detectably labeled prion chimera can be added to the solid support under complex forming conditions. In this instance, the detectably labeled second antibody is specific for pathogenic prions (*e.g.*, is generated using a prion chimera). Complexes formed between the first antibody and/or the prion chimera, a pathogenic prion from the sample, and the second detectably labeled antibody and/or detectably labeled prion chimera can be detected.

The antibodies and chimeras of the invention may also be used in competition assays. Means of detection can be used to identify when an antigen weakly binding to an antibody specific to pathogenic prions is displaced by a pathogenic prion.

For instance, a first antibody specific to pathogenic prions (or a prion chimera) is adsorbed onto a solid support. Preferably, the antibody is generated according to methods described herein. The solid support is combined with a detectably labeled first antigen or ligand so that the first antigen ligand is bound to the first antibody (or prion chimera). The binding affinity of the first antibody (or prion chimera) and the detectably labeled first antigen or ligand (or detectably labeled prion chimera) is weaker than the binding affinity of the first antibody (prion chimera) and a pathogenic prion. Then, the support is combined with a sample (*e.g.*, a biological sample) suspected of containing a pathogenic prion, under conditions that allow the pathogenic prion, when present in the sample, to bind to the first

antibody (or prion chimera) and replace the first detectably labeled antigen or ligand. Finally, complexes formed between the first antibody (or prion chimera) and pathogenic prions from the biological sample can be detected. Such detection can be accomplished by, for instance, comparing the amount of detectably labeled first antigen or ligand present on the support prior to exposure to the biological sample with the amount present after exposure to the biological sample.

The above-described assay reagents, including the antibodies and/or prion chimeras described above, can be provided in kits, with suitable instructions and other necessary reagents, in order to conduct immunoassays as described above. Where the antibody and/or prion chimera is adsorbed onto a solid support, the kit may additionally or alternatively comprise such antibodies and/or prion chimeras adsorbed onto one or more solid supports. The kit may further contain suitable positive and negative controls, as described above. The kit can also contain, depending on the particular immunoassay used, suitable labels ad other packaged reagents and materials (i.e., wash buffers and the like). Standard immunoassays, such as those described above, can be conducted using these kits.

In still further embodiments, the invention is directed to solid supports comprising an antibody specific to pathogenic prions, an antibody capable of binding to both pathogenic and nonpathogenic prions, and/or a prion chimera. Methods of producing these solid supports are also provided, for example by (a) providing a solid support; and (b) binding thereto one or more moieties selected from the group consisting of an antibody specific to pathogenic prions, an antibody capable of binding to both pathogenic and nonpathogenic prions, and a prion chimera.

The polyclonal or monoclonal antibodies may further be used to isolate disease form proteins by immunoaffinity columns. The antibodies can be affixed to a solid support by, for example, adsorption or by covalent linkage so that the antibodies retain their immunoselective activity. Optionally, spacer groups may be included so that the antigen binding site of the antibody remains accessible. The immobilized antibodies can then be used to bind the target from a biological sample, such as blood or plasma. The bound proteins or complexes are recovered from the column matrix by, for example, a change in pH.

Biological samples that can be tested according to the invention include any sample amenable to an antibody assay, including blood and tissue samples.

VI. Immunogenic Compositions

The invention further relates to immunogenic compositions comprising the antibodies and prion chimeras (and polynucleotides encoding the chimeras and/or antibodies) and methods of using these compositions in therapeutic and prophylactic vaccines for the treatment or prevention of prion-related diseases. Thus, the antibodies, prion chimeras (and polynucleotides encoding these antibodies and/or chimeras) can also be used in immunogenic (*e.g.*, vaccine) compositions, individually or in combination, for prophylactic (i.e., to prevent pathogenesis) or therapeutic (to treat disease following infection) purposes.

The immunogenic compositions (*e.g.*, vaccines) can comprise mixtures of one or more of the antibodies, chimeras and/or polynucleotides. These molecules may be obtained from a variety of sources, for example, recombinantly produced protein, synthetically produced proteins, etc. The vaccine may also be administered in conjunction with other antigens and immunoregulatory agents, for example, immunoglobulins, cytokines, lymphokines, and chemokines, including but not limited to IL-2, modified IL-2 (cys125-ser125), GM-CSF, IL-12, alpha- or gamma-interferon, IP-10, MIP1 and RANTES. The vaccines may be administered as polypeptides or, alternatively, as naked nucleic acid vaccines (*e.g.*, DNA), using viral vectors (*e.g.*, retroviral vectors, adenoviral vectors, adeno-associated viral vectors, alphaviral vectors) or non-viral vectors (*e.g.*, liposomes, particles coated with nucleic acid or protein).

The immunogenic compositions may also comprise a mixture of protein and nucleic acid, which in turn may be delivered using the same or different modalities and/or vehicles. The vaccine may be given more than once (*e.g.*, a "prime" administration followed by one or more "boosts") to achieve the desired effects. The same composition can be administered as the prime and as the one or more boosts. Alternatively, different compositions can be used for priming and boosting.

In certain embodiments, the methods of the invention comprise administering an immunogenic composition comprising a prion chimera, an antibody specific for pathogenic prions and/or polynucleotides encoding these chimeras or antibodies to an animal. The immunogenic compositions used in the invention preferably comprise an immunologically effective amount of these components. An "immunologically effective amount" is an amount sufficient to allow the mammal to raise an immune response to a prion protein, preferably a pathogenic prion. Still more preferably, the immune response is directed against the β-helical conformation of pathogenic prions.

The immune response generally involves the production of antibodies specific to the prion chimera and the pathogenic prion conformation. The amount of antibodies produced

will vary depending on several factors including the animal used, the presence of an adjuvant, etc.

The immunogenic compositions of the invention may further comprise one or more adjuvants. Adjuvants suitable for use in the invention include one or more of the following:

- E.coli heat-labile enterotoxin ("LT"), or detoxified mutants thereof, such as the K63 or R72 mutants;
- cholera toxin ("CT"), or detoxified mutants thereof;
- microparticles (i.e., a particle of ~100nm to ~150μm in diameter, more preferably ~200nm to ~30μm in diameter, and most preferably ~500nm to ~10μm in diameter) formed from materials that are biodegradable and non-toxic (*e.g.* a poly(α-hydroxy acid), a polyhydroxybutyric acid, a polyorthoester, a polyanhydride, a polycaprolactone *etc.*);
- a polyoxyethylene ether or a polyoxyethylene ester (*see* International patent application WO 99/52549);
- a polyoxyethylene sorbitan ester surfactant in combination with an octoxynol (*see* International patent application WO 01/21207) or a polyoxyethylene alkyl ether or ester surfactant in combination with at least one additional non-ionic surfactant such as an octoxynol (*see* International patent application WO 01/21152);
- chitosan (*e.g.* International patent application WO 99/27960)
- an immunostimulatory oligonucleotide (*e.g.* a CpG oligonucleotide) and a saponin (*see* International patent application WO 00/62800)
- immunostimulatory double stranded RNA.
- aluminum compounds (*e.g.* aluminum hydroxide, aluminum phosphate, aluminum hydroxyphosphate, oxyhydroxide, orthophosphate, sulfate *etc.* (*e.g.* see chapters 8 & 9 of *Vaccine design: the subunit and adjuvant approach*, eds. Powell & Newman, Plenum Press 1995 (ISBN 0-306-44867-X) (hereinafter "*Vaccine design*"), or mixtures of different aluminum compounds, with the compounds taking any suitable form (*e.g.* gel, crystalline, amorphous *etc.*), and with adsorption being preferred;
- MF59 (5% Squalene, 0.5% Tween 80, and 0.5% Span 85, formulated into submicron particles using a microfluidizer) (*see* Chapter 10 of *Vaccine design*; *see also* International patent application WO 90/14837);
- liposomes (see Chapters 13 and 14 of *Vaccine design*);
- ISCOMs (*see* Chapter 23 of *Vaccine design*);

- SAF, containing 10% Squalane, 0.4% Tween 80, 5% pluronic-block polymer L121, and thr-MDP, either microfluidized into a submicron emulsion or vortexed to generate a larger particle size emulsion (*see* Chapter 12 of *Vaccine design*);

- Ribi™ adjuvant system (RAS), (Ribi Immunochem) containing 2% Squalene, 0.2% Tween 80, and one or more bacterial cell wall components from the group consisting of monophosphorylipid A (MPL), trehalose dimycolate (TDM), and cell wall skeleton (CWS), preferably MPL + CWS (Detox™);

- saponin adjuvants, such as QuilA or QS21 (*see* Chapter 22 of *Vaccine design*), also known as Stimulon™;

- ISCOMs, which may be devoid of additional detergent (International patent application WO 00/07621);

- complete Freund's adjuvant (CFA) and incomplete Freund's adjuvant (IFA);

- cytokines, such as interleukins (*e.g.* IL-1, IL-2, IL-4, IL-5, IL-6, IL-7, IL-12, *etc.*), interferons (*e.g.* interferon-γ), macrophage colony stimulating factor, tumor necrosis factor, *etc.* (see Chapters 27 & 28 of *Vaccine design*);

- monophosphoryl lipid A (MPL) or 3-O-deacylated MPL (3dMPL) (*e.g.* chapter 21 of *Vaccine design*);

- combinations of 3dMPL with, for example, QS21 and/or oil-in-water emulsions (European patent applications 0835318, 0735898 and 0761231);

- oligonucleotides comprising CpG motifs (*see* Krieg (2000) *Vaccine*, 19:618 – 622; Krieg (2001) *Curr. Opin. Mol. Ther.*, 2001, 3:15 – 24; WO 96/02555, WO 98/16247, WO 98/18810, WO 98/40100, WO 98/55495, WO 98/37919 and WO 98/52581, etc.) *i.e.* containing at least one CG dinucleotide,

- a polyoxyethylene ether or a polyoxyethylene ester (International patent application WO 99/52549);

- a polyoxyethylene sorbitan ester surfactant in combination with an octoxynol (International patent application WO 01/21207) or a polyoxyethylene alkyl ether or ester surfactant in combination with at least one additional non-ionic surfactant such as an octoxynol (International patent application WO 01/21152);

- an immunostimulatory oligonucleotide (*e.g.* a CpG oligonucleotide) and a saponin (International patent application WO 00/62800);

- an immunostimulant and a particle of metal salt (International patent application WO 00/23105);

- a saponin and an oil-in-water emulsion (International patent application WO 99/11241); and

- a saponin (*e.g.* QS21) + 3dMPL + IL-12 (optionally + a sterol) (International patent application WO 98/57659).

Muramyl peptides include, but are not limited to, N-acetyl-muramyl-L-threonyl-D-isoglutamine (thr-MDP), N-acteyl-normuramyl-L-alanyl-D-isogluatme (nor-MDP), N-acetylmuramyl-L-alanyl-D-isogluatminyl-L-alanine-2-(l'-2'-dipalmitoyl-*sn*-glycero-3-huydroxyphosphoryloxy)-ethylamine (MTP-PE), etc.

Other adjuvants suitable for mucosal or parenteral administration are also available (*e.g.* see chapter 7 of *Vaccine design: the subunit and adjuvant approach*, eds. Powell & Newman, Plenum Press 1995 (ISBN 0-306-44867-X).

Mutants of LT are preferred adjuvants (*e.g.*, mucosal adjuvants), in particular the "K63" and "R72" mutants (*e.g.* see International patent application WO 98/18928), as these result in an enhanced immune response.

Microparticles are also useful as adjuvants. These are preferably derived from a poly(α-hydroxy acid), in particular, from a poly(lactide) ("PLA"), a copolymer of D,L-lactide and glycolide or glycolic acid, such as a poly(D,L-lactide-co-glycolide) ("PLG" or "PLGA"), or a copolymer of D,L-lactide and caprolactone. The microparticles may be derived from any of various polymeric starting materials that have a variety of molecular weights and, in the case of the copolymers such as PLG, a variety of lactide:glycolide ratios, the selection of which will be largely a matter of choice, depending in part on the coadministered antigen. The prions, antibodies, polynucleotides and/or adjuvants of the invention may be entrapped within the microparticles, or may be adsorbed to them. Entrapment within PLG microparticles is preferred. PLG microparticles are discussed in further detail in Morris et al., (1994), Vaccine, 12:5 – 11, in chapter 13 of Mucosal Vaccines, eds. Kiyono et al., Academic Press 1996 (ISBN 012410587), and in chapters 16 & 18 of *Vaccine design: the subunit and adjuvant approach*, eds. Powell & Newman, Plenum Press 1995 (ISBN 0-306-44867-X).

LT mutants may advantageously be used in combination with microparticle-entrapped antigen, resulting in significantly enhanced immune responses.

Aluminum compounds and MF59 are preferred adjuvants for parenteral use. Typically, the vaccine compositions are prepared as injectables, either as liquid solutions or suspensions; solid forms suitable for solution in, or suspension in, liquid vehicles prior to

injection may also be prepared. The preparation also may be emulsified or encapsulated in liposomes for enhanced adjuvant effect, as discussed above.

Pharmaceutically acceptable salts can also be used in compositions of the invention, for example, mineral salts such as hydrochlorides, hydrobromides, phosphates, or sulfates, as well as salts of organic acids such as acetates, proprionates, malonates, or benzoates. Especially useful protein substrates are serum albumins, keyhole limpet hemocyanin, immunoglobulin molecules, thyroglobulin, ovalbumin, tetanus toxoid, and other proteins well known to those of skill in the art.

Compositions of the invention can also contain liquids or excipients, such as water, saline, glycerol, dextrose, ethanol, or the like, singly or in combination, as well as substances such as wetting agents, emulsifying agents, or pH buffering agents. A carrier is optionally present which is a molecule that does not itself induce the production of antibodies harmful to the individual receiving the composition. Suitable carriers are typically large, slowly metabolized macromolecules such as proteins, polysaccharides, polylactic acids, polyglycolic acids, polymeric amino acids, amino acid copolymers, lipid aggregates (such as oil droplets or liposomes), and inactive virus particles. Such carriers are well known to those of ordinary skill in the art. Furthermore, one or more polypeptides in the immunogenic composition may be conjugated to a bacterial toxoid, such as toxoid from diphtheria, tetanus, cholera, etc.

Vaccine compositions will typically comprise a therapeutically effective amount of the molecules (chimeras) or nucleotide sequences encoding the same, antibodies directed to these molecules and any other of the above-mentioned components, as needed. By "therapeutically effective amount" is meant an amount that will induce a protective and/or therapeutic immunological response in the uninfected, infected or unexposed individual to whom it is administered. Such a response will generally result in the development in the subject of a secretory, cellular and/or antibody-mediated immune response to the vaccine. Usually, such a response includes but is not limited to one or more of the following effects; the production of antibodies from any of the immunological classes, such as immunoglobulins A, D, E, G or M; the proliferation of B and T lymphocytes; the provision of activation, growth and differentiation signals to immunological cells; expansion of helper T cell, suppressor T cell, and/or cytotoxic T cell. Preferably, the antibodies of the invention are specific to the pathogenic prion conformation. Still more preferably, the antibodies are specific to the β-helical region of pathogenic prions. Accordingly, the invention also comprises a composition comprising an effective amount of antibodies specific to pathogenic prions, for example generated using the methods described herein. The invention further

comprises a composition comprising an effective amount of antibodies specific to the β-helical region of a pathogenic prion. A "therapeutically effective amount" will fall in a relatively broad range that can be determined through routine trials. The exact amount necessary will vary depending on the subject being treated; the age and general condition of the individual to be treated; the capacity of the individual's immune system to synthesize antibodies; the degree of protection desired; the severity of the condition being treated; the particular vaccine selected and its mode of administration, among other factors.

The immunogenic (*e.g.,* vaccine) compositions of the invention are preferably pharmaceutically acceptable and pharmacologically acceptable. In particularly, the compositions are preferably not biologically or otherwise undesirable, *i.e.,* the material may be administered to an individual in a formulation or composition without causing any undesirable biological effects or interacting in a deleterious manner with any of the components of the composition in which it is contained.

VI.A. Delivery

The immunogenic compositions of the invention may be administered in a single dose, or as part of an administration regime. Nucleic acids, antibodies and/or peptides may be administered may be administered by any suitable modality including, but not limited to intramuscularly, intramucosally, subcutaneously, intradermally, transdermally, intravaginally, intrarectally, orally and/or intravenously. The dosage regime may include priming and boosting doses, which may be administered mucosally, parenterally, or various combinations thereof.

In certain embodiments, one or more components of the immunogenic compositions are administered parenterally or mucosally. Suitable routes of parenteral administration include intramuscular (IM), subcutaneous, intravenous, intraperitoneal, intradermal, transcutaneous, and transdermal (*see e.g.*, International patent application WO 98/20734) routes, as well as delivery to the interstitial space of a tissue. Suitable routes of mucosal administration include oral, intranasal, intragastric, pulmonary, intestinal, rectal, ocular and vaginal routes. The immunogenic composition may be adapted for mucosal administration. For instance, where the composition is for oral administration, it may be in the form of tablets or capsules, optionally enteric-coated, liquid, transgenic plants, etc. Where the composition is for intranasal administration, it may be in the form of a nasal spray, nasal drops, gel or powder. Dosage treatment may be a single dose schedule or a multiple dose schedule.

For example, the nucleic acid-containing immunogenic compositions (*e.g.*, vaccines) may be accomplished with or without viral vectors, as described above, by injection using either a conventional syringe or a gene gun, such as the Accell® gene delivery system (PowderJect Technologies, Inc., Oxford, England). Delivery of DNA into cells of the epidermis is particularly preferred as this mode of administration provides access to skin-associated lymphoid cells and provides for a transient presence of DNA in the recipient.

As noted above, polynucleotide sequences coding for the above-described molecules (chimeras and/or antibodies) can be obtained using recombinant methods, such as by screening cDNA and genomic libraries from cells expressing the gene, or by deriving the gene from a vector known to include the same. Furthermore, the desired gene can be isolated directly from cells and tissues containing the same, using standard techniques, such as phenol extraction and PCR of cDNA or genomic DNA. See, e.g., Sambrook et al., *supra*, for a description of techniques used to obtain and isolate DNA. The gene of interest can also be produced synthetically, rather than cloned. The nucleotide sequence can be designed with the appropriate codons for the particular amino acid sequence desired. In general, one will select preferred codons for the intended host in which the sequence will be expressed. The complete sequence is assembled from overlapping oligonucleotides prepared by standard methods and assembled into a complete coding sequence. See, e.g., Edge, *Nature* (1981) 292:756; Nambair et al., *Science* (1984) 223:1299; Jay et al., *J. Biol. Chem.* (1984) 259:6311; Stemmer, W.P.C., (1995) *Gene* 164:49-53.

The gene sequence encoding the desired chimera or antibody can be inserted into a vector. Insertions can be made within the coding sequence or at either end of the coding sequence. Vectors may include control elements operably linked to the coding sequence, which allow for the expression of the gene *in vivo* in the subject species. For example, typical promoters for mammalian cell expression include the SV40 early promoter, a CMV promoter such as the CMV immediate early promoter, the mouse mammary tumor virus LTR promoter, the adenovirus major late promoter (Ad MLP), and the herpes simplex virus promoter, among others. Other nonviral promoters, such as a promoter derived from the murine metallothionein gene, will also find use for mammalian expression. Typically, transcription termination and polyadenylation sequences will also be present, located 3' to the translation stop codon. Preferably, a sequence for optimization of initiation of translation, located 5' to the coding sequence, is also present. Examples of transcription terminator/polyadenylation signals include those derived from SV40, as described in Sambrook et al., *supra*, as well as a bovine growth hormone terminator sequence.

Enhancer elements may also be used herein to increase expression levels of the mammalian constructs. Examples include the SV40 early gene enhancer, as described in Dijkema et al., *EMBO J.* (1985) 4:761, the enhancer/promoter derived from the long terminal repeat (LTR) of the Rous Sarcoma Virus, as described in Gorman et al., *Proc. Natl. Acad. Sci. USA* (1982b) 79:6777 and elements derived from human CMV, as described in Boshart et al., *Cell* (1985) 41:521, such as elements included in the CMV intron A sequence.

The constructs may be uni-cistronic or, alternatively, multi-cistronic cassettes (e.g., bi-cistronic cassettes) can be constructed allowing expression of multiple antigens from a single mRNA using the EMCV IRES, or the like.

Once complete, the constructs can be used for nucleic acid immunization using standard gene delivery protocols. Methods for gene delivery are known in the art. See, e.g., U.S. Patent Nos. 5,399,346, 5,580,859, 5,589,466. Polynucleotides can be delivered either directly to the vertebrate subject or, alternatively, delivered *ex vivo*, to cells derived from the subject and the cells reimplanted in the subject.

A number of viral based systems have been developed for gene transfer into mammalian cells. For example, retroviruses provide a convenient platform for gene delivery systems. Selected sequences can be inserted into a vector and packaged in retroviral particles using techniques known in the art. The recombinant virus can then be isolated and delivered to cells of the subject either *in vivo* or *ex vivo*. A number of retroviral systems have been described (U.S. Patent No. 5,219,740; Miller and Rosman, *BioTechniques* (1989) 7:980-990; Miller, A.D., *Human Gene Therapy* (1990) 1:5-14; Scarpa et al., *Virology* (1991) 180:849-852; Burns et al., *Proc. Natl. Acad. Sci. USA* (1993) 90:8033-8037; and Boris-Lawrie and Temin, *Cur. Opin. Genet. Develop.* (1993) 3:102-109.

A number of adenovirus vectors have also been described. Unlike retroviruses which integrate into the host genome, adenoviruses persist extrachromosomally thus minimizing the risks associated with insertional mutagenesis (Haj-Ahmad and Graham, *J. Virol.* (1986) 57:267-274; Bett et al., *J. Virol.* (1993) 67:5911-5921; Mittereder et al., *Human Gene Therapy* (1994) 5:717-729; Seth et al., *J. Virol.* (1994) 68:933-940; Barr et al., *Gene Therapy* (1994) 1:51-58; Berkner, K.L. *BioTechniques* (1988) 6:616-629; and Rich et al., *Human Gene Therapy* (1993) 4:461-476).

Molecular conjugate vectors, such as the adenovirus chimeric vectors described in Michael et al., *J. Biol. Chem.* (1993) 268:6866-6869 and Wagner et al., *Proc. Natl. Acad. Sci. USA* (1992) 89:6099-6103, can also be used for gene delivery.

Additionally, various adeno-associated virus (AAV) vector systems have been developed for gene delivery. AAV vectors can be readily constructed using techniques well known in the art. See, e.g., U.S. Patent Nos. 5,173,414 and 5,139,941; International Publication Nos. WO 92/01070 (published 23 January 1992) and WO 93/03769 (published 4 March 1993); Lebkowski et al., *Molec. Cell. Biol.* (1988) 8:3988-3996; Vincent et al., *Vaccines 90* (1990) (Cold Spring Harbor Laboratory Press); Carter, B.J. *Current Opinion in Biotechnology* (1992) 3:533-539; Muzyczka, N. *Current Topics in Microbiol. and Immunol.* (1992) 158:97-129; Kotin, R.M. *Human Gene Therapy* (1994) 5:793-801; Shelling and Smith, *Gene Therapy* (1994) 1:165-169; and Zhou et al., *J. Exp. Med.* (1994) 179:1867-1875.

Additional viral vectors that will find use for delivering the nucleic acid molecules encoding the antigens of interest include those derived from the pox family of viruses, including vaccinia virus and avian poxvirus. An example of a pox virus system for enterically administered recombinant poxvirus vaccines is described by Small, Jr., P.A., et al. (U.S. Patent No. 5,676,950, issued October 14, 1997, herein incorporated by reference). Vaccinia virus recombinants expressing the genes can be constructed as follows. The DNA is first inserted into an appropriate vector so that it is adjacent to a vaccinia promoter and flanking vaccinia DNA sequences, such as the sequence encoding thymidine kinase (TK). This vector is then used to transfect cells that are simultaneously infected with vaccinia. Homologous recombination serves to insert the vaccinia promoter plus the gene encoding the coding sequences of interest into the viral genome. The resulting TK-recombinant can be selected by culturing the cells in the presence of 5-bromodeoxyuridine and by picking viral plaques resistant thereto.

Alternatively, avipoxviruses, such as the fowlpox and canarypox viruses, can also be used to deliver the genes. Recombinant avipox viruses, expressing immunogens from mammalian pathogens, are known to confer protective immunity when administered to non-avian species. The use of an avipox vector is particularly desirable in human and other mammalian species since members of the avipox genus can only productively replicate in susceptible avian species and therefore are not infective in mammalian cells. Methods for producing recombinant avipoxviruses are known in the art and employ genetic recombination, as described above with respect to the production of vaccinia viruses. See, e.g., WO 91/12882; WO 89/03429; and WO 92/03545. Picornavirus-derived vectors can also be used. (See, e.g., U.S. Patent Nos. 5,614,413 and 6,063,384).

Members of the Alphavirus genus, such as, but not limited to, vectors derived from the Sindbis, Semliki Forest, and Venezuelan Equine Encephalitis viruses, will also find use as

viral vectors for delivering the polynucleotides of the present invention. For a description of Sindbis-virus derived vectors useful for the practice of the instant methods, see, Dubensky et al., *J. Virol.* (1996) 70:508-519; and International Publication Nos. WO 95/07995 and WO 96/17072; as well as, Dubensky, Jr., T.W., et al., U.S. Patent No. 5,843,723, issued December 1, 1998, and Dubensky, Jr., T.W., U.S. Patent No. 5,789,245, issued August 4, 1998, both herein incorporated by reference. See, also, U.S. Patent Nos. 6,342,372; 6,329,201 and International Publication WO 01/92552.

A vaccinia based infection/transfection system can be conveniently used to provide for inducible, transient expression of the coding sequences of interest in a host cell. In this system, cells are first infected *in vitro* with a vaccinia virus recombinant that encodes the bacteriophage T7 RNA polymerase. This polymerase displays exquisite specificity in that it only transcribes templates bearing T7 promoters. Following infection, cells are transfected with the polynucleotide of interest, driven by a T7 promoter. The polymerase expressed in the cytoplasm from the vaccinia virus recombinant transcribes the transfected DNA into RNA that is then translated into protein by the host translational machinery. The method provides for high level, transient, cytoplasmic production of large quantities of RNA and its translation products. See, e.g., Elroy-Stein and Moss, *Proc. Natl. Acad. Sci. USA* (1990) 87:6743-6747; Fuerst et al., *Proc. Natl. Acad. Sci. USA* (1986) 83:8122-8126.

As an alternative approach to infection with vaccinia or avipox virus recombinants, or to the delivery of genes using other viral vectors, an amplification system can be used that will lead to high level expression following introduction into host cells. Specifically, a T7 RNA polymerase promoter preceding the coding region for T7 RNA polymerase can be engineered. Translation of RNA derived from this template will generate T7 RNA polymerase that in turn will transcribe more template. Concomitantly, there will be a cDNA whose expression is under the control of the T7 promoter. Thus, some of the T7 RNA polymerase generated from translation of the amplification template RNA will lead to transcription of the desired gene. Because some T7 RNA polymerase is required to initiate the amplification, T7 RNA polymerase can be introduced into cells along with the template(s) to prime the transcription reaction. The polymerase can be introduced as a protein or on a plasmid encoding the RNA polymerase. For a further discussion of T7 systems and their use for transforming cells, see, e.g., International Publication No. WO 94/26911; Studier and Moffatt, *J. Mol. Biol.* (1986) 189:113-130; Deng and Wolff, *Gene* (1994) 143:245-249; Gao et al., *Biochem. Biophys. Res. Commun.* (1994) 200:1201-1206; Gao and Huang, *Nuc. Acids*

Res. (1993) 21:2867-2872; Chen et al., *Nuc. Acids Res.* (1994) 22:2114-2120; and U.S. Patent No. 5,135,855.

Polynucleotides encoding chimeras and/or antibodies as described herein can also be delivered without a viral vector. For example, the construct can be packaged in liposomes prior to delivery to the subject or to cells derived therefrom. Lipid encapsulation is generally accomplished using liposomes that are able to stably bind or entrap and retain nucleic acid. The ratio of condensed DNA to lipid preparation can vary but will generally be around 1:1 (mg DNA:micromoles lipid), or more of lipid. For a review of the use of liposomes as carriers for delivery of nucleic acids, see, Hug and Sleight, *Biochim. Biophys. Acta.* (1991) 1097:1-17; Straubinger et al., in *Methods of Enzymology* (1983), Vol. 101, pp. 512-527.

Liposomal preparations for use in the present invention include cationic (positively charged), anionic (negatively charged) and neutral preparations, with cationic liposomes particularly preferred. Cationic liposomes have been shown to mediate intracellular delivery of plasmid DNA (Felgner et al., *Proc. Natl. Acad. Sci. USA* (1987) 84:7413-7416); mRNA (Malone et al., *Proc. Natl. Acad. Sci. USA* (1989) 86:6077-6081); and purified transcription factors (Debs et al., *J. Biol. Chem.* (1990) 265:10189-10192), in functional form.

Cationic liposomes are readily available. For example, N[1-2,3-dioleyloxy)propyl]-N,N,N-triethyl-ammonium (DOTMA) liposomes are available under the trademark Lipofectin, from GIBCO BRL, Grand Island, NY. (See, also, Felgner et al., *Proc. Natl. Acad. Sci. USA* (1987) 84:7413-7416). Other commercially available lipids include (DDAB/DOPE) and DOTAP/DOPE (Boerhinger). Other cationic liposomes can be prepared from readily available materials using techniques well known in the art. See, e.g., Szoka et al., *Proc. Natl. Acad. Sci. USA* (1978) 75:4194-4198; PCT Publication No. WO 90/11092 for a description of the synthesis of DOTAP (1,2-bis(oleoyloxy)-3-(trimethylammonio)propane) liposomes.

Similarly, anionic and neutral liposomes are readily available, such as, from Avanti Polar Lipids (Birmingham, AL), or can be easily prepared using readily available materials. Such materials include phosphatidyl choline, cholesterol, phosphatidyl ethanolamine, dioleoylphosphatidyl choline (DOPC), dioleoylphosphatidyl glycerol (DOPG), dioleoylphoshatidyl ethanolamine (DOPE), among others. These materials can also be mixed with the DOTMA and DOTAP starting materials in appropriate ratios. Methods for making liposomes using these materials are well known in the art.

The liposomes can comprise multilammelar vesicles (MLVs), small unilamellar vesicles (SUVs), or large unilamellar vesicles (LUVs). The various liposome-nucleic acid

complexes are prepared using methods known in the art. See, e.g., Straubinger et al., in METHODS OF IMMUNOLOGY (1983), Vol. 101, pp. 512-527; Szoka et al., *Proc. Natl. Acad. Sci. USA* (1978) 75:4194-4198; Papahadjopoulos et al., *Biochim. Biophys. Acta* (1975) 394:483; Wilson et al., *Cell* (1979) 17:77); Deamer and Bangham, *Biochim. Biophys. Acta* (1976) 443:629; Ostro et al., *Biochem. Biophys. Res. Commun.* (1977) 76:836; Fraley et al., *Proc. Natl. Acad. Sci. USA* (1979) 76:3348); Enoch and Strittmatter, *Proc. Natl. Acad. Sci. USA* (1979) 76:145); Fraley et al., *J. Biol. Chem.* (1980) 255:10431; Szoka and Papahadjopoulos, *Proc. Natl. Acad. Sci. USA* (1978) 75:145; and Schaefer-Ridder et al., *Science* (1982) 215:166.

The DNA, antibodies and/or protein chimera(s) described herein can also be delivered in cochleate lipid compositions similar to those described by Papahadjopoulos et al., *Biochem. Biophys. Acta.* (1975) 394:483-491. See, also, U.S. Patent Nos. 4,663,161 and 4,871,488.

The vaccine compositions (or components thereof) may also be encapsulated, adsorbed to, or associated with, particulate carriers. Such carriers present multiple copies of a selected antigen to the immune system and promote trapping and retention of antigens in local lymph nodes. The particles can be phagocytosed by macrophages and can enhance antigen presentation through cytokine release. Examples of particulate carriers include those derived from polymethyl methacrylate polymers, as well as microparticles derived from poly(lactides) and poly(lactide-co-glycolides), known as PLG. See, e.g., Jeffery et al., *Pharm. Res.* (1993) 10:362-368; McGee JP, et al., *J Microencapsul.* **14**(2):197-210, 1997; O'Hagan DT, et al., *Vaccine* **11**(2):149-54, 1993. Suitable microparticles may also be manufactured in the presence of charged detergents, such as anionic or cationic detergents, to yield microparticles with a surface having a net negative or a net positive charge. For example, microparticles manufactured with anionic detergents, such as hexadecyltrimethylammonium bromide (CTAB), i.e. CTAB-PLG microparticles, adsorb negatively charged macromolecules, such as DNA. (see, e.g., Int'l Application Number PCT/US99/17308).

Furthermore, other particulate systems and polymers can be used for the *in vivo* or *ex vivo* delivery. For example, polymers such as polylysine, polyarginine, polyornithine, spermine, spermidine, as well as conjugates of these molecules, are useful for transferring a nucleic acid of interest. Similarly, DEAE dextran-mediated transfection, calcium phosphate precipitation or precipitation using other insoluble inorganic salts, such as strontium phosphate, aluminum silicates including bentonite and kaolin, chromic oxide, magnesium

silicate, talc, and the like, will find use with the present methods. See, e.g., Felgner, P.L., *Advanced Drug Delivery Reviews* (1990) 5:163-187, for a review of delivery systems useful for gene transfer. Peptoids (Zuckerman, R.N., et al., U.S. Patent No. 5,831,005, issued November 3, 1998, herein incorporated by reference) may also be used for delivery of a construct of the present invention.

Additionally, biolistic delivery systems employing particulate carriers such as gold and tungsten, are especially useful for delivering polynucleotides of the present invention. The particles are coated with the polynucleotide(s) and/or polypeptides to be delivered and accelerated to high velocity, generally under a reduced atmosphere, using a gun powder discharge from a "gene gun." For a description of such techniques, and apparatuses useful therefore, see, e.g., U.S. Patent Nos. 4,945,050; 5,036,006; 5,100,792; 5,179,022; 5,371,015; and 5,478,744. Also, needle-less injection systems can be used (Davis, H.L., et al, *Vaccine* 12:1503-1509, 1994; Bioject, Inc., Portland, OR).

The methods of the invention further comprise treating or preventing a prion-relating disease by administering to an animal a composition comprising an effective amount of the antibodies of the invention.

Methods of treatment may combine both immunogenic compositions and antibody compositions. Accordingly the invention comprises a method for treating or preventing a prion-related disease comprising administering an immunogenic composition comprising an immunologically effective amount of a prion chimera and administering an effective amount of antibodies specific to pathogenic prions. The immunogenic composition and the antibodies may be administered together or separately. The invention further comprises a composition comprising an immunogenic composition comprising an immunologically effective amount of a prion chimera and further comprising an effective amount of antibodies specific to pathogenic prions.

The chimeras, polynucleotides and/or antibodies used in the invention can be administered to an animal. Animals suitable for use in the methods of the invention include humans and other primates, including non-human primates such as chimpanzees, and other apes and monkey species; farm animals such as cattle, sheep, pigs, goats and horses, domestic animals such as dogs and cats; laboratory animals including rodents such as mice, rats and guinea pigs; birds, including domestic, wild and game birds such as chickens, turkeys and other gallinaceous birds, ducks, geese and the like. Animals suitable for use in the invention can be of any age, including both adult and newborn. Transgenic animals can also be used in

the invention. See generally, Prusiner "Prions" *Proc. Natl. Acad. Sci. USA* (1998) 95:13363-13383 for a discussion of transgenic animals currently used to study prion-related diseases.

The compositions of the invention can be used to treat or prevent prion-related diseases. Such prion-related diseases include a disease cause in whole or in part by a pathogenic prion particle (PrP^{Sc}). Prion-related diseases include scrapie, bovine spongiform encephalopathies (BSE), mad cow disease, feline spongiform encephalopathies, kuru, Creutzfeldt-Jakob Disease (CJD), Gerstmann-Strassler-Scheinker Disease (GSS), and fatal familial insomnia (FFI).

EXAMPLES

The following examples are offered for illustrative purposes only, and are not intended to limit the scope of the present invention in any way.

Example 1: Cloning

GCA and P69 DNA sequences were made synthetically as follows. P.69 pertactin DNA sequences were amplified directly from *Bordatella pertussis* strains W28 and SA1 using standard PCR techniques. PrP DNA sequences were also amplified from genomic DNA using standard PCR techniques. The chimeras were then created by taking advantage of unique restriction sites within the natural sequences. All constructs were made in the non-expressing plasmid vector pUC18 and later excised and religated into the Stratagene pET3a plasmid vector. Nucleic acids encoding a leader sequence (*e.g.*, a tpa leader sequence) may also be included in the constructs. (See, *e.g.*, FIGs. 28-31 (SEQ ID NO:155-182) for exemplary sequences of DNA constructs encoding prion chimeras).

Example 2: Expression and Purification

BL21 cells were transformed with each the pET31 expression vectors prepared as described in Example 1. Cells grown in 1L flasks from 25ml overnight starter cultures in the presence of ampicillin as per the directions associated with the pET3a vector. Expression was induced at log phase (approximately at OD = 400) using 0.4 mM IPTG and allowed to proceed for 4 hours. Supernatant and pellet samples from small scale (10ml) expression experiment were also evaluated on SDS-PAGE and by Western Blotting (probed with anti-His_6 antibodies) to determine solubility and expression levels.

A. Purification

Bacteria were cracked open by 2 rounds of freezing and thawing at –80 degrees Celsius and room temperature respectively. The bacteria were further disrupted with 5 one minute rounds of sonication with a Branson Sonifier Microtip near the energy limit for the microtip. The bacteria were then centrifuged for 45 minutes at 16,000 rpm in a Beckman JA20 rotor. For GCA chimera purification the supernatant was decanted and saved for the next step in the purification. For P.69 purification the supernatant was discarded and the pellet was saved for the next step in the purification.

A.1. P.69 Purification

P.69 was purified from inclusion bodies. The pellet fraction following centrifugation was resolublized in 6M guanadine-HCl overnight. The solublized material was slowly adjusted to 2M guanadine-HCl and then dialyzed against PBS, changing the buffer every hour for five hours. Precipitate was collected by centrifugation for 30 minutes at 16,000 rpm in a Beckman JA20 rotor. Expression levels and resolublization was evaluated by running SDS-PAGE gels of the supernatant and pellet fractions. In general the protein would be ~80% pure following resolublization. The protein when soluble was then further purified by passing the supernatant over a Ni-NTA resin (Qiagen) and eluting with a gradient of increasing imidizole concentration. All purifiable constructs eluted between 200 and 400 mM imidizole. Fractions were analyzed using SDS-PAGE. Protein samples were further purified by reducing the pooled GCA containing fractions in 10mM DTT followed by size exclusion chromatography and buffer exchange using a Superdex 75 column (Pharmacia) in PBS. This protein was then used directly for a variety of experiments including CD spectrometery, direct binding assays with PrP^{Sc}, and as an antigen for the generation of polyclonal antibodies in rabbits (Josman, LLC).

A.2 GCA Purification

GCA was purified to a high degree of homogeneity (>95%) by passing the supernatant over a Ni-NTA resin (Qiagen) and eluting with a gradient of increasing imidizole concentration. All purifiable constructs eluted between 200 and 400 mM imidizole. Fractions were analyzed using SDS-PAGE. Protein samples were further purified by reducing the pooled GCA containing fractions in 10mM DTT followed by size exclusion chromatography and buffer exchange using a Superdex 75 column (Pharmacia) in PBS. This protein was then used directly for a variety of experiments including CD spectrometery,

direct binding assays with PrP^{Sc}, and as an antigen for the generation of polyclonal antibodies in rabbits (Josman, LLC).

Example 3: Chimera Binding Assays

Chimeras are tested for their ability to directly bind to prion proteins by incubating either prion infected mouse brains (both treated and untreated with proteinase K) or normal mouse brains with fusion protein coated nickel resin over night. The resin is washed extensively, and bound protein is eluted from the resin by incubating it with SDS-PAGE gel loading buffer containing 500 mM imidizole. The eluted protein is detected by Western blotting with an antibody that recognizes denatured PrP.

Example 4: Antibody Production

The following provides an example of a protocol that can be used to generate antibodies to the prion chimeras of the invention.

Mice are immunized with a composition comprising a prion chimera either IM (intramuscular) or IP (intraperitoneal) on day 0, followed by 2 – 5 boosts at intervals of not more frequently than every 2 weeks. Blood is collected before the first immunization and then 7 days following each boost to monitor the humoral response to the antigen. 6 orbital eye bleeds are taken from each animal (three from each eye) of approximately 0.2 mls or less per bleed. The final boost is delivered by IV (intravenous) injection. Three days after the final boost, mice are euthanized by exposure to CO_2 or isofluorane followed by cervical dislocation. Spleens are then harvested for hybridoma production.

Freund's adjuvant, complete, is used as an adjuvant for the first injection followed by Incomplete Freund's adjuvant for the remaining infections, except for the IV infection. The IV injections are prepared in saline.

SEQ ID NOS. 14 – 153 are non-limiting examples several prion chimeras which can be used in this procedure.

Example 5: Antibody Production

The following provides an example of a protocol that can be used to generate antibodies to the prion chimeras of the invention.

Mice are immunized with a composition comprising a polynucleotide encoding for a prion chimera either IM (intramuscular) or IP (intraperitoneal) on day 0, followed by 2 – 5 boosts at intervals of not more frequently than every 2 weeks. Blood is collected before the

first immunization and then 7 days following each boost to monitor the humoral response to the antigen. 6 orbital eye bleeds are taken from each animal (three from each eye) of approximately 0.2 mls or less per bleed. The final boost is delivered by IV (intravenous) injection. Three days after the final boost, mice are euthanized by exposure to CO_2 or isofluorane followed by cervical dislocation. Spleens are then harvested for hybridoma production.

Adjuvants suitable for polynucleotide administration can optionally be used in the administered compositions. For instance, the polynucleotides can be adsorbed onto a microparticle, such as a poly(lactide-co-glycolide) (PLG) microparticle.

Polynucleotides encoding for SEQ ID NOS. 14 – 125 are examples of polynucleotides that can be used in this procedure.

Table 1 summarizes selected characteristics of exemplary constructs encoding various exemplary prion chimeras. Sequence Identification Numbers are given relative to the chimera encoded by the construct. "pAbs" refers to generation of polyclonal antibodies. "TBD" refers to experiments to be done.

Table 1

Seq ID No.	Construct Encodes	Expression Tested	Over expressed	Soluble or Pellet	Purified	pABs	Other Comments
3	Full length P69 His	Yes	No	Pellet	Yes	Yes	
4	Full Length GCAHis	Yes	No	NA	Yes	Yes	
8	N-terminally truncated P69 control A His	Yes	No	Pellet	TBD	TBD	
9	His C-terminally truncated P69 control B	Yes	No	Pellet	TBD	TBD	
10	N-terminally truncated GCA Control A His	Yes	No	Soluble	Yes	TBD	
11	HisC-terminally truncated GCA Control B	Yes	No	NA	No	TBD	
14	$HuPrP_{135-155}$P69A-1 His	Yes	Yes	Pellet	TBD	TBD	
15	$HuPrP_{135-155}$P69A-2 His	Yes	Yes	Pellet	TBD	TBD	
16	$HuPrP_{135-155}$P69A-3 His	Yes	Yes	Pellet	TBD	TBD	
17	$HuPrP_{135-155}$P69A-4 His	Yes	Yes	Pellet	TBD	TBD	
18	$HuPrP_{135-155}$P69A-5 His	Yes	Yes	Pellet	TBD	TBD	
19	$HuPrP_{135-155}$P69A-6 His	Yes	Yes	Pellet	TBD	TBD	
20	$HuPrP_{135-155}$P69A-7 His	Yes	Yes	Pellet	TBD	TBD	
21	$HuPrP_{135-155}$P69-8 His	Yes	Yes	Pellet	TBD	TBD	
22	$MoPrP_{135-155}$P69A1His	Yes	Yes	Pellet	Yes	TBD	Can be folded and solubilized from inclusion bodies
23	$MoPrP_{135-155}$P69A2 His	Yes	Yes	Pellet	Yes	Yes	Can be folded and solubilized from inclusion bodies
24	$MoPrP_{135-155}$P69A3 His	Yes	Yes	Pellet	Yes	TBD	Can be folded and solubilized from inclusion bodies

25	MoPrP$_{135-155}$P69A4 His	Yes	Yes	Pellet	Yes	TBD	Can be folded and solubilized from inclusion bodies.
26	MoPrP$_{135-155}$P69A5 His	Yes	Yes	Pellet	Yes	TBD	Can be folded and solubilized from inclusion bodies.
27	MoPrP$_{135-155}$P69A6 His	Yes	Yes	Pellet	Yes	TBD	Can be folded and solubilized from inclusion bodies.
28	MoPrP$_{135-155}$P69A7 His	Yes	Yes	Pellet	Yes	TBD	Can be folded and solubilized from inclusion bodies.
29	MoPrP$_{135-155}$P69A 8 His	Yes	Yes	Pellet	Yes	TBD	Can be folded and solubilized from inclusion bodies.
30	His P69BHuPrP$_{135-155}$-1	Yes	Yes	Pellet	TBD	TBD	
31	His P69BHuPrP$_{135-155}$-2	Yes	Yes	Pellet	TBD	TBD	
32	His P69BHuPrP$_{135-155}$-3	Yes	Yes	Pellet	TBD	TBD	
33	His P69BHuPrP$_{135-155}$-4	Yes	Yes	Pellet	TBD	TBD	
34	His P69BHuPrP$_{135-155}$-5	Yes	Yes	Pellet	TBD	TBD	
35	His P69BHuPrP$_{135-155}$-6	Yes	Yes	Pellet	TBD	TBD	
36	His P69BHuPrP$_{135-155}$-7	Yes	Yes	Pellet	TBD	TBD	
37	His P69BHuPrP$_{135-155}$-8	Yes	Yes	Pellet	TBD	TBD	
38	HisP69BMoPrP$_{135-155}$-1	Yes	Yes	Pellet	TBD	TBD	Can be folded and solubilized from inclusion bodies.

39	HisP69BMoPrP$_{135-155}$-2	Yes	Yes	Pellet	TBD	TBD	Can be folded and solubilized from inclusion bodies.
40	HisP69BMoPrP$_{135-155}$-3	Yes	Yes	Pellet	TBD	TBD	Can be folded and solubilized from inclusion bodies.
41	HisP69BMoPrP$_{135-155}$-4	Yes	Yes	Pellet	TBD	TBD	Can be folded and solubilized from inclusion bodies.
42	HisP69BMoPrP$_{135-155}$-5	Yes	Yes	Pellet	TBD	TBD	Can be folded and solubilized from inclusion bodies.
43	HisP69BMoPrP$_{135-155}$-6	Yes	Yes	Pellet	TBD	TBD	Can be folded and solubilized from inclusion bodies.
44	HisP69BMoPrP$_{135-155}$-7	Yes	Yes	Pellet	TBD	TBD	Can be folded and solubilized from inclusion bodies.
45	HisP69BMoPrP$_{135-155}$-8	Yes	Yes	Pellet	TBD	TBD	Can be folded and solubilized from inclusion bodies.
46	HuPrP$_{135-155}$GCAA-1His	Yes	Yes	Soluble	TBD	TBD	
47	HuPrP$_{135-155}$GCAA-2His	Yes	Yes	Soluble	TBD	TBD	

48	$HuPrP_{135-155}$GCAA-3His	Yes	Yes	Soluble	TBD	TBD	
49	$HuPrP_{135-155}$GCAA-4His	Yes	Yes	Soluble	TBD	TBD	
50	$HuPrP_{135-155}$GCAA-5His	Yes	Yes	Soluble	TBD	TBD	
51	$HuPrP_{135-155}$GCAA-6His	Yes	Yes	Soluble	TBD	TBD	
52	$MoPrP_{135-155}$GCAA-1His	Yes	Yes	Soluble	Yes	TBD	
53	$MoPrP_{135-155}$GCAA-2 His	Yes	Yes	Soluble	Yes	Yes	Binds to mouse prion protein *in vitro*
54	$MoPrP_{135-155}$GCAA-3 His	Yes	Yes	Soluble	Yes	Yes	Binds to mouse prion protein *in vitro*
55	$MoPrP_{135-155}$GCAA-4 His	Yes	Yes	Soluble	Yes	Yes	Binds to mouse prion protein *in vitro*
56	$MoPrP_{135-155}$GCAA-5 His	Yes	Yes	Soluble	Yes	Yes	Binds to mouse prion protein *in vitro*
57	$MoPrP_{135-155}$GCAA-6 His	Yes	Yes	Soluble	Yes	TBD	
58	HisGCABHuPrP$_{135-155}$-1	Yes	No	TBD	TBD	TBD	
59	HisGCABHuPrP$_{135-155}$-2	Yes	No	TBD	TBD	TBD	
60	HisGCABHuPrP$_{135-155}$-3	Yes	No	TBD	TBD	TBD	
61	HisGCABHuPrP$_{135-155}$-4	Yes	No	TBD	TBD	TBD	
62	HisGCABHuPrP$_{135-155}$-5	Yes	No	TBD	TBD	TBD	
63	HisGCABHuPrP$_{135-155}$-6	Yes	No	TBD	TBD	TBD	
64	HisGCABMoPrP$_{135-155}$-1	Yes	No	TBD	TBD	TBD	

65	HisGCAAMoPrP$_{135-155}$-2	Yes	No	TBD	TBD	TBD	
66	HisGCABMoPrP$_{135-155}$-3.	Yes	No	TBD	TBD	TBD	
67	HisGCABMoPrP$_{135-155}$-4	Yes	No	TBD	TBD	TBD	
68	HisGCABMoPrP$_{135-155}$-5	Yes	No	TBD	TBD	TBD	
69	HisGCABMoPrP$_{135-155}$-6	Yes	No	TBD	TBD	TBD	
185	HisGCABnoLHuPrP$_{135-155}$-1	Yes	TBD	TBD	TBD	TBD	
186	HisGCABnoLHuPrP$_{135-155}$-2	Yes	TBD	TBD	TBD	TBD	
187	HisGCABnoLHuPrP$_{135-155}$-3	Yes	TBD	TBD	TBD	TBD	
188	HisGCABnoLHuPrP$_{135-155}$-4	Yes	TBD	TBD	TBD	TBD	
189	HisGCABnoLHuPrP$_{135-155}$-5	Yes	TBD	TBD	TBD	TBD	
190	HisGCABnoLHuPrP$_{135-155}$-6	Yes	TBD	TBD	TBD	TBD	

WHAT IS CLAIMED IS:

1. A method of generating antibodies specific to a pathogenic prion, the method comprising the step of administering to an animal a prion chimera, wherein the chimera comprises

a prion protein or a fragment or derivative thereof; and

a non-prion, β-helical protein or a fragment or derivative thereof.

2. The method of embodiment 1, wherein the prion protein or fragment or derivative thereof has a β-helical conformation of a pathogenic prion.

3. The method of embodiment 1, wherein the prion protein comprises the amino acid sequence set forth in SEQ ID NO:1 or SEQ ID NO:2.

4. The method of embodiment 1, wherein the fragment of the prion protein comprises the amino acid sequence set forth in SEQ ID NO:6, SEQ ID NO:12, SEQ ID NO:7 or SEQ ID NO:13.

5. The method of embodiment 1, wherein one or more amino acids of the β-helix of the β-helical protein is replaced with the prion protein or fragment or derivative thereof.

6. The method of embodiment 1, wherein the non-prion β-helical protein a left handed helical protein or a right handed helical protein.

7. The method of embodiment 6, wherein the non-prion β-helical protein is derived from pertactin or γ carbonic anhydrase.

8. The method of embodiment 7, wherein the non-prion β-helical protein is derived from P69 pertactin.

9. The method of embodiment 7, wherein the non-prion protein comprises the amino acid sequence set forth in SEQ ID NO:3, SEQ ID NO:8, SEQ ID NO:9, SEQ ID NO:4, SEQ ID NO:10, SEQ ID NO:11, SEQ ID NO:183 or SEQ ID NO:184.

10. The method of embodiment 1, wherein the prion chimera further comprises a tag sequence.

11. The method of embodiment 10, wherein the tag sequence is a histidine tag sequence.

12. The method of embodiment 11, wherein the histidine tag sequence comprises the amino acid sequence set forth in SEQ ID NO. 5 or SEQ ID NO:6.

13. The method of embodiment 1, further comprising the step of isolating the specific antibodies from the animal.

14. The method of embodiment 1, wherein the prion chimera is not pathogenic.

15. The method of embodiment 1, wherein the prion chimera is selected from the group consisting of SEQ ID NO:14-153 and SEQ ID NO:185-208.

16. The method of any of embodiments 1-15, wherein the prion chimera is encoded by a polynucleotide.

17. An antibody specific to a prion chimera, wherein the prion chimera comprises a prion protein or a fragment or derivative thereof; and a non-prion, β-helical protein or a fragment or derivative thereof.

18. The antibody of embodiment 17 generated by the method of any of embodiments 1 through 15.

19. The antibody of embodiment 17, wherein the antibody is a monoclonal antibody.

20. The antibody of embodiment 17, wherein the antibody is a polyclonal antibody.

21. The antibody of embodiment 17, wherein the antibody specific to the β-helical region of a pathogenic prion.

22. A polynucleotide encoding an antibody according to embodiment 17.

23. A method for detecting the presence of a pathogenic prion in a biological sample comprising:

(a) exposing the biological sample suspected of containing a pathogenic prion to a prion chimera comprising a prion protein or a fragment or derivative thereof and a non-prion, β-helical protein or a fragment or derivative thereof; and

(b) detecting the presence or absence of the prion chimera bound to the pathogenic prion, if any, in the sample.

24. A method for detecting the presence of a pathogenic prion in a biological sample, comprising:

(a) exposing the biological sample to a first antibody specific for a prion chimera comprising a prion protein or a fragment or derivative thereof and a non-prion, β-helical protein or a fragment or derivative thereof; and

(b) detecting the presence or absence of the first antibody bound to a pathogenic prion.

25. A method for detecting a pathogenic prion in a biological sample, comprising:

(a) providing a solid support comprising a first antibody bound thereto, wherein the first antibody is specific for a prion chimera comprising a prion protein or a fragment or derivative thereof and a non-prion, β-helical protein or a fragment or derivative thereof;

(b) exposing the solid support to a biological sample under conditions which allow pathogenic prions, when present in the biological sample, to bind to the first antibody;

(c) exposing the solid support to a detectably labeled second antibody specific to pathogenic prions under conditions which allow the second antibody to bind to pathogenic prions bound by the first antibody; and

(d) detecting complexes formed between the first antibody, a pathogenic prion from the biological sample and the second antibody, thereby detecting the presence of the pathogenic prion in the biological sample.

26. A method for detecting the presence of a pathogenic prion in a biological sample comprising:

(a) providing a solid support comprising a first antibody bound thereto, wherein the first antibody recognizes pathogenic and non-pathogenic prions;

(b) exposing the solid support to a biological sample under conditions which allow prion proteins, when present in the biological sample, to bind to the first antibody;

(c) exposing the solid support to a detectably labeled second antibody specific for a prion chimera comprising a prion protein or a fragment or derivative thereof and a non-prion, β-helical protein or a fragment or derivative thereof or a detectably labeled prion chimera that binds to pathogenic prions; and

(d) detecting complexes formed between the first antibody, a pathogenic prion from the biological sample, and the second antibody or the prion chimera.

27. A method for detecting the presence of a pathogenic prion in a biological sample comprising:

(a) providing a solid support comprising a first antibody bound thereto, wherein the first antibody is specific for a prion chimera comprising a prion protein or a fragment or derivative thereof and a non-prion, β-helical protein or a fragment or derivative thereof;

(b) combining the solid support with a detectably labeled first ligand, wherein the first antibody's binding affinity to the detectably labeled first ligand is weaker than the first antibody's binding affinity to a pathogenic prion;

(c) combining a biological sample with the solid support under conditions which allow a pathogenic prion, when present in the biological sample, to bind to the first antibody and replace the first ligand;

(d) detecting complexes formed between the first antibody and the pathogenic prion from the biological sample.

28. The method of any of embodiments 24-27, wherein the antibody specific for the prion chimera is generated according to any of the methods of embodiments 1-15.

29. The method of any of embodiments 25-27, wherein the solid support is selected from the group consisting of nitrocellulose, polystyrene latex, polyvinyl fluoride, diazotized paper, nylon membranes, activated beads, and magnetically responsive beads.

30. The method of any of embodiments 23-27, wherein the biological sample is selected from the group consisting of organs, whole blood, blood fractions, plasma, cerebrospinal fluid (CSF), urine, tears, tissue, organs, and biopsies.

31. The method of embodiment 30, wherein the biological sample is blood.

32. A solid support comprising at least one antibody specific to a prion chimera or at least one prion chimera, wherein the prion chimera comprises a prion protein or a fragment or derivative thereof and a non-prion, β-helical protein or a fragment or derivative thereof bound thereto.

33. The solid support of embodiment 32, wherein the antibody is directed against a β-helical region of a pathogenic prion.

34. A solid support for use in an immunoassay, the solid support comprising at least one antibody specific to pathogenic and nonpathogenic prions bound thereto.

35. A kit for detecting the presence of a pathogenic prion in a biological sample comprising:

(a) a solid support according to any of embodiments 32-34; and

(b) other necessary reagents and, optionally, positive and negative controls.

36. An immunogenic composition comprising a prion chimera and an adjuvant, wherein the prion chimera comprises a prion protein or a fragment or derivative thereof and a non-prion β-helical protein or a fragment or derivative thereof.

37. The immunogenic composition of embodiment 36, wherein the prion protein comprises a fragment of a prion protein which can adopt a β-helical conformation of a pathogenic prion protein.

38. The immunogenic composition of embodiment 36, wherein the prion protein comprises the amino acid sequence set forth in SEQ ID NO:1 or SEQ ID NO:2.

39. The immunogenic composition of embodiment 36, wherein the fragment of the prion protein comprises the amino acid sequence set forth in SEQ ID NO:6, SEQ ID NO:12, SEQ ID NO:7 or SEQ ID NO:13.

40. The immunogenic composition of embodiment 36, wherein one or more amino acids of the β-helix of the β-helical protein is replaced with the prion protein or fragment or derivative thereof.

41. The immunogenic composition of embodiment 36, wherein the non-prion β-helical protein a left handed helical protein or a right handed helical protein.

42. The immunogenic composition of embodiment 41, wherein the non-prion β-helical protein is derived from pertactin or γ carbonic anhydrase.

43. The immunogenic composition of embodiment 42, wherein the non-prion β-helical protein is derived from P69 pertactin.

44. The immunogenic composition of embodiment 42, wherein the non-prion protein comprises the amino acid sequence set forth in SEQ ID NO:3, SEQ ID NO:8, SEQ ID NO:9, SEQ ID NO:4, SEQ ID NO:10, SEQ ID NO:11, SEQ ID NO:183 or SEQ ID NO:184.

45. The immunogenic composition of embodiment 36, wherein the prion chimera is selected from the group consisting of SEQ ID NO:14-153, and SEQ ID NO:185-208.

46. An immunogenic composition comprising a polynucleotide encoding prion chimera and an adjuvant, wherein the prion chimera encoded by the polynucleotide comprises a prion protein or a fragment or derivative thereof and a non-prion β-helical protein or a fragment or derivative thereof.

47. The immunogenic composition of embodiment 46, wherein the polynucleotide is selected from the group consisting of SEQ ID NO:155-182.

48. A method of raising an immune response to a pathogenic prion comprising administering to an animal an immunologically effective amount of the immunogenic composition according to any of embodiments 36-45.

49. A method of raising an immune response to a pathogenic prion comprising administering to an animal an immunologically effective amount of the immunogenic composition according to embodiment 46 or embodiment 47.

50. The method of embodiment 49, wherein the polynucleotide is incorporated into a gene delivery vehicle and the gene delivery vehicle is selected from the group consisting of a viral vector, a non-viral vector, a particular carrier, and a liposome preparation.

51. The method of any of embodiments 48-50, wherein the subject is a mammal.

52. The method of embodiment 51, wherein the mammal is a human.

53. The method of any of embodiment 48-50, wherein the immunogenic composition is administered intramuscularly, intramucosally, intranasally, subcutaneously, intradermally, transdermally, intravaginally, intrarectally, orally or intravenously.

54. A method of inducing an immune response in a subject comprising

(a) administering a first composition comprising an immunogenic composition according to embodiment 46 or embodiment 47 in a priming step and

(b) administering a second composition comprising an immunogenic composition according to embodiments 36-45, as a booster, in an amount sufficient to induce an immune response in the subject.

55. The method of embodiment 54, wherein the first composition or second composition further comprise an adjuvant.

56. A method of raising an immune response to a pathogenic prion comprising administering to an animal an antibody specific for a prion chimera comprising a prion protein or a fragment or derivative thereof and a non-prion, β-helical protein or a fragment or derivative thereof.

57. The method of embodiment 56, wherein the antibody is generated according to any of the methods of embodiments 1-15.

58. A method of treating or preventing a pathogenic prion related disease comprising administering to an animal an immunologically effective amount of a prion chimera, wherein the chimera comprises a prion protein or a fragment or derivative thereof and a non-prion β-helical protein or a fragment or derivative thereof.

FIGURE 1A

HUMAN PRION AMINO ACID SEQUENCES

Amino Acid Sequence of a Full Length Human Prion Protein:

SEQ ID NO. 1: M A N L G C W M L V L F V A T W S D L G L C K K R P K P G G W N T G G S R Y P G Q G S P G G N R Y P P Q G G G G W G Q P H G G G W G Q P H G G G W G Q P H G G G W G Q P H G G G W G Q G G G T H S Q W N K P S K P K T N M K H M A G A A A A G A V V G G L G G Y M L G S A M S R P I I H F G S D Y E D R Y Y R E N M H R Y P N Q V Y Y R P M D E Y S N Q N N F V H D C V N I T I K Q H T V T T T T K G E N F T E T D V K M M E R V V E Q M C I T Q Y E R E S Q A Y Y Q R G S S M V L F S S P P V I L L I S F L I F L I V G

Amino Acid Sequence of Fragment of Human Prion Protein:

Human PrP (135 – 155)

SEQ ID NO. 6: R P I I H F G S D Y E D R Y Y R E N M H R

Amino Acid Sequence of a Fragment of Human Prion Protein:
Human PrP (126 – 154)

SEQ ID NO. 12: G Y M L G S A M S R P I I H F G S D Y E D R Y Y R E N M H

FIGURE 1B

MOUSE PRION AMINO ACID SEQUENCES

Amino Acid Sequence of a Full Length Mouse Prion Protein:

SEQ ID. NO. 2: M A N L G Y W L L A L F V T M W T D V G L C K K R P K P G G W N T G G S R Y P G Q G S P G G N R Y P P Q G G T W G Q P H G G G W G Q P H G G S W G Q P H G G S W G Q P H G G G W G Q G G G T H N Q W N K P S K P K T N L K H V A G A A A A G A V V G G L G G Y M L G S A M S R P M I H F G N D W E D R Y Y R E N M Y R Y P N Q V Y Y R P V D Q Y S N Q N N F V H D C V N I T I K Q H T V T T T T K G E N F T E T D V K M M E R V V E Q M C V T Q Y Q K E S Q A Y Y D G R R S S S T V L F S S P P V I L L I S F L I F L I V G

Amino Acid Sequence of a Fragment of Mouse Prion Protein:
Mouse PrP (135 – 155)

SEQ ID NO. 7: R P M I H F G N D W E D R Y Y R E N M Y R

Amino Acid Sequence of a Fragment of Mouse Prion Protein:
Mouse PrP (126 – 154)

SEQ ID NO. 13: G Y M L G S A M S R P M I H F G N D W E D R Y Y R E N M Y

FIGURE 2A

P69 AMINO ACID SEQUENCES

Full length Amino Acid Sequence of P69

SEQ ID NO. 3: MNMSLSRIVKAAPLRRTTLAMALGALGA
APAAHADWNNQSIVKTGERQHGIHIQGSDPGGVRTASGT
TIKVSGRQAQGILLENPAAELQFRNGSVTSSGQLSDDGIR
RFLGTVTVKAGKLVADHATLANVGDTWDDDGIALYVAGE
QAQASIADSTLQGAGGVQIERGANVTVQRSAIVDGGLHIG
ALQSLQPEDLPPSRVVLRDTNVTAVPASGAPAAVSVLGAS
ELTLDGGHITGGRAAGVAAMQGAVVHLQRATIRRGDAPA
GGAVPGGAVPGGFGP GGFGPGGFGPGGFGPVLDGWYGV
DVSGSSVELAQSIVEAPELGAAIRVGRGARVTVSGGSLSAP
HGNVIETGGARRFAPQAAPLSITLQAGAHAQGKALLYRV
LPEPVKLTLTGGADAQGDIVATELPSIPGTSIGPLDVALAS
QARWTGATRAVDSLSIDNATWVMTDNSNVGALRLASDGS
VDFQQPAEAGRFKVLTVNTLAGSGLFRMNVFADLGLSDK
LVVMQDASGQHRLWVRNSGSEPASANTLLLVQTPLGSAA
TFTLANKDGKVDIGTYRYRLAANGNGQWSLVGAKAPPAP
KPAPQPGPQPPQPPQPQPEAPAPQPPAGRELSAAANAAVN
TGGVGLASTLWYAESNALSKRLGELRLNPDAGGAWGRGF
AQRQQLDNRAGRRFDQKVAGFELGADHAVAVAGGRWHL
GGLAGYTRGDRGFTGDGGGHTDSVHVGGYATYIADSGFY
LDATLRASRLENDFKVAGSDGYAVKGKYRTHGVGASLEA
GRRFTHADGWFLEPQAELAVFRAGGGAYRAANGLRVRDE
GGSSVLGRLGLEVGKRIELAGGRQVQPYIKASVLQEFDGA
GTVHTNGIAHRTELRGTRAELGLGMAAALGRGHSLYASY
EYSKGPKLAMPWTFHAGYRYSW

FIGURE 2B

P69 AMINO ACID SEQUENCES

P69 Control A

SEQ ID NO. 8: DWNNQSIVKTGERQHGIHIQGSDPGGVRTASG
TTIKVSGRQAQGILLENPAAELQFRNGSVTSSGQLSDDGIRR
FLGTVTVKAGKLVADHATLANVGDTWDDDGIALYVAGEQA
QASIADSTLQGAGGVQIERGANVTVQRSAIVDGGLHIGALQS
LQPEDLPPSRVVLRDTNVTAVPASGAPAAVSVLGASELTLD
GGHITGGRAAGVAAMQGAVVHLQRATIRRGDAPAGGAVPG
GAVPGGFGPGGGFGPGGGFGPGGGFGPVLDGWYGVDVSGSSVEL
AQSIVEAPELGAAIRVGRGARVTVSGGSLSAPHGNVIETGGA
RRFAPQAAPLSITLQAGAHAQGKALLYRVLPEPVKLTLTGG
ADAQGDIVATELPSIPGTSIGPLDVALASQARWTGATRAVDS
LSIDNATWVMTDNSNVGALRLASDGSVDFQQPAEAGRFKVL
TVNTLAGSGLFRMNVFADLGLSDKLVVMQDASGQHRLWVR
NSGSEPASANTLLLVQTPLGSAATFTLANKDGKVDIGTYRY
RLAANGNGQWSLVGAKAPP

P 69 Control B:

SEQ ID. NO. 9: MNMSLSRIVKAAPLRRTTLAMALGALGAAPA
AHADWNNQSIVKTGERQHGIHIQGSDPGGVRTASGTTIKVS
GRQAQGILLENPAAELQFRNGSVTSSGQLSDDGIRRFLGTVT
VKAGKLVADHATLANVGDTWDDDGIALYVAGEQAQASIAD
STLQGAGGVQIERGANVTVQRSAIVDGGLHIGALQSLQPED
LPPSRVVLRDTNVTAVPASGAPAAVSVLGASELTLDGGHIT
GGRAAGVAAMQGAVVHLQRATIRRGDAPAGGAVPGGAVPG
GFGPGGGFGPGGGFGPGGGFGPVLDGWYGVDVSGSSVELAQSIV
EAPELGAAIRVGRGARVTVSGGSLSAPHGNVIETGGARRFAP
QAAPLSITLQAGAHAQGKALLYRVLPEPVKLTLTGGADAQG
DIVATELPSIPGTSIGPLDVALASQARWTGATRAVDSLSIDN
ATWVMTDNSNVGALRLASDGSVDFQQPAEAGRFKVLTVNT
LA

FIGURE 3A

PRION CHIMERAS USING P69 CONTROL A AND HUMAN PrP (135 – 155)

P69/Hu135-155 Chimera No. 1: Human PrP (135 – 155) – P69 Control A – His Tag

SEQ ID NO. 14:

MRPIIHFGSDYEDRYYRENMHRDWNNQSIVKTGERQHGIHIQGSDPGGVRTASG
TTIKVSGRQAQGILLENPAAELQFRNGSVTSSGQLSDDGIRRFLGTVTVKAGKLV
ADHATLANVGDTWDDDGIALYVAGEQAQASIADSTLQGAGGVQIERGANVTVQ
RSAIVDGGLHIGALQSLQPEDLPPSRVVLRDTNVTAVPASGAPAAVSVLGASELT
LDGGHITGGRAAGVAAMQGAVVHLQRATIRRGDALAGGAVPGGAVPGGAVPG
GFGPGGFGPVLDGWYGVDVSGSSVELAQSIVEAPELGAAIRVGRGARVTVPGGS
LSAPHGNVIETGGARRFAPQAAPLSITLQAGAHAQGKALLYRVLPEPVKLTLTG
GADAQGDIVATELPSIPGTSIGPLDVALASQARWTGATRAVDSLSIDNATWVMT
DNSNVGALRLASDGSVDFQQPAEAGRFKVLTVNTLAGSGLFRMNVFADLGLSD
KLVVMQDASGQHRLWVRNSGSEPASANTLLLVQTPLGSAATFTLANKDGKVDI
GTYRYRLAANGNGQWSLVGAKAPPGGHHHHHH

P69/Hu135-155 Chimera No. 2: Human PrP (135 – 155) – P69 Control A – His Tag

SEQ ID NO. 15:

MRPIIHFGSDYEDRYYRENMHRWNNQSIVKTGERQHGIHIQGSDPGGVRTASGT
TIKVSGRQAQGILLENPAAELQFRNGSVTSSGQLSDDGIRRFLGTVTVKAGKLV
ADHATLANVGDTWDDDGIALYVAGEQAQASIADSTLQGAGGVQIERGANVTVQ
RSAIVDGGLHIGALQSLQPEDLPPSRVVLRDTNVTAVPASGAPAAVSVLGASELT
LDGGHITGGRAAGVAAMQGAVVHLQRATIRRGDALAGGAVPGGAVPGGAVPG
GFGPGGFGPVLDGWYGVDVSGSSVELAQSIVEAPELGAAIRVGRGARVTVPGGS
LSAPHGNVIETGGARRFAPQAAPLSITLQAGAHAQGKALLYRVLPEPVKLTLTG
GADAQGDIVATELPSIPGTSIGPLDVALASQARWTGATRAVDSLSIDNATWVMT
DNSNVGALRLASDGSVDFQQPAEAGRFKVLTVNTLAGSGLFRMNVFADLGLSD
KLVVMQDASGQHRLWVRNSGSEPASANTLLLVQTPLGSAATFTLANKDGKVDI
GTYRYRLAANGNGQWSLVGAKAPPGGHHHHHH

FIGURE 3B

PRION CHIMERAS USING P69 CONTROL A AND HUMAN PrP (135 – 155)

P69/Hu135-155 Chimera No. 3: Human PrP (135 – 155) – P69 Control A – His Tag

SEQ ID NO. 16:

MRPIIHFGSDYEDRYYRENMHRNNQSIVKTGERQHGIHIQGSDPGGVRTASGTTI KVSGRQAQGILLENPAAELQFRNGSVTSSGQLSDDGIRRFLGTVTVKAGKLVAD HATLANVGDTWDDDGIALYVAGEQAQASIADSTLQGAGGVQIERGANVTVQRS AIVDGGLHIGALQSLQPEDLPPSRVVLRDTNVTAVPASGAPAAVSVLGASELTLD GGHITGGRAAGVAAMQGAVVHLQRATIRRGDALAGGAVPGGAVPGGAVPGGF GPGGFGPVLDGWYGVDVSGSSVELAQSIVEAPELGAAIRVGRGARVTVPGGSLS APHGNVIETGGARRFAPQAAPLSITLQAGAHAQGKALLYRVLPEPVKLTLTGGA DAQGDIVATELPSIPGTSIGPLDVALASQARWTGATRAVDSLSIDNATWVMTDNS NVGALRLASDGSVDFQQPAEAGRFKVLTVNTLAGSGLFRMNVFADLGLSDKLV VMQDASGQHRLWVRNSGSEPASANTLLLVQTPLGSAATFTLANKDGKVDIGTY RYRLAANGNGQWSLVGAKAPPGGHHHHHH

P69/Hu135-155 Chimera No. 4: Human PrP (135 – 155) – P69 Control A – His Tag

SEQ ID NO. 17:

MRPIIHFGSDYEDRYYRENMHRNQSIVKTGERQHGIHIQGSDPGGVRTASGTTI KVSGRQAQGILLENPAAELQFRNGSVTSSGQLSDDGIRRFLGTVTVKAGKLVAD HATLANVGDTWDDDGIALYVAGEQAQASIADSTLQGAGGVQIERGANVTVQRS AIVDGGLHIGALQSLQPEDLPPSRVVLRDTNVTAVPASGAPAAVSVLGASELTLD GGHITGGRAAGVAAMQGAVVHLQRATIRRGDALAGGAVPGGAVPGGAVPGGF GPGGFGPVLDGWYGVDVSGSSVELAQSIVEAPELGAAIRVGRGARVTVPGGSLS APHGNVIETGGARRFAPQAAPLSITLQAGAHAQGKALLYRVLPEPVKLTLTGGA DAQGDIVATELPSIPGTSIGPLDVALASQARWTGATRAVDSLSIDNATWVMTDNS NVGALRLASDGSVDFQQPAEAGRFKVLTVNTLAGSGLFRMNVFADLGLSDKLV VMQDASGQHRLWVRNSGSEPASANTLLLVQTPLGSAATFTLANKDGKVDIGTY RYRLAANGNGQWSLVGAKAPPGGHHHHHH

FIGURE 3C

PRION CHIMERAS USING P69 CONTROL A AND HUMAN PrP (135 – 155)

P69/Hu135-155 Chimera No. 5: Human PrP (135 – 155) – P69 Control A – His Tag

SEQ ID NO. 18:
MRPIIHFGSDYEDRYYRENMHRQSIVKTGERQHGIHIQGSDPGGVRTASGTTIK
VSGRQAQGILLENPAAELQFRNGSVTSSGQLSDDGIRRFLGTVTVKAGKLVADH
ATLANVGDTWDDDGIALYVAGEQAQASIADSTLQGAGGVQIERGANVTVQRSAI
VDGGLHIGALQSLQPEDLPPSRVVLRDTNVTAVPASGAPAAVSVLGASELTLDG
GHITGGRAAGVAAMQGAVVHLQRATIRRGDALAGGAVPGGAVPGGAVPGGFG
PGGFGPVLDGWYGVDVSGSSVELAQSIVEAPELGAAIRVGRGARVTVPGGSLSA
PHGNVIETGGARRFAPQAAPLSITLQAGAHAQGKALLYRVLPEPVKLTLTGGAD
AQGDIVATELPSIPGTSIGPLDVALASQARWTGATRAVDSLSIDNATWVMTDNSN
VGALRLASDGSVDFQQPAEAGRFKVLTVNTLAGSGLFRMNVFADLGLSDKLVV
MQDASGQHRLWVRNSGSEPASANTLLLVQTPLGSAATFTLANKDGKVDIGTYR
YRLAANGNGQWSLVGAKAPPGGHHHHHH

P69/Hu135-155 Chimera No. 6: Human PrP (135 – 155) – P69 Control A – His Tag

SEQ ID NO. 19:
MRPIIHFGSDYEDRYYRENMHRSIVKTGERQHGIHIQGSDPGGVRTASGTTIKVS
GRQAQGILLENPAAELQFRNGSVTSSGQLSDDGIRRFLGTVTVKAGKLVADHAT
LANVGDTWDDDGIALYVAGEQAQASIADSTLQGAGGVQIERGANVTVQRSAIV
DGGLHIGALQSLQPEDLPPSRVVLRDTNVTAVPASGAPAAVSVLGASELTLDGG
HITGGRAAGVAAMQGAVVHLQRATIRRGDALAGGAVPGGAVPGGAVPGGFGP
GGFGPVLDGWYGVDVSGSSVELAQSIVEAPELGAAIRVGRGARVTVPGGSLSAP
HGNVIETGGARRFAPQAAPLSITLQAGAHAQGKALLYRVLPEPVKLTLTGGAD
AQGDIVATELPSIPGTSIGPLDVALASQARWTGATRAVDSLSIDNATWVMTDNSN
VGALRLASDGSVDFQQPAEAGRFKVLTVNTLAGSGLFRMNVFADLGLSDKLVV
MQDASGQHRLWVRNSGSEPASANTLLLVQTPLGSAATFTLANKDGKVDIGTYR
YRLAANGNGQWSLVGAKAPPGGHHHHHH

FIGURE 3D

PRION CHIMERAS USING P69 CONTROL A AND HUMAN PrP (135 – 155)

P69/Hu135-155 Chimera No. 7: Human PrP (135 – 155) – P69 Control A – His Tag

SEQ ID NO. 20:

MRPIIHFGSDYEDRYYRENMHRIVKTGERQHGIHIQGSDPGGVRTASGTTIKVS
GRQAQGILLENPAAELQFRNGSVTSSGQLSDDGIRRFLGTVTVKAGKLVADHAT
LANVGDTWDDDGIALYVAGEQAQASIADSTLQGAGGVQIERGANVTVQRSAIV
DGGLHIGALQSLQPEDLPPSRVVLRDTNVTAVPASGAPAAVSVLGASELTLDGG
HITGGRAAGVAAMQGAVVHLQRATIRRGDALAGGAVPGGAVPGGAVPGGFGP
GGFGPVLDGWYGVDVSGSSVELAQSIVEAPELGAAIRVGRGARVTVPGGSLSAP
HGNVIETGGARRFAPQAAPLSITLQAGAHAQGKALLYRVLPEPVKLTLTGGAD
AQGDIVATELPSIPGTSIGPLDVALASQARWTGATRAVDSLSIDNATWVMTDNSN
VGALRLASDGSVDFQQPAEAGRFKVLTVNTLAGSGLFRMNVFADLGLSDKLVV
MQDASGQHRLWVRNSGSEPASANTLLLVQTPLGSAATFTLANKDGKVDIGTYR
YRLAANGNGQWSLVGAKAPPGGHHHHHH

P69/Hu135-155 Chimera No. 8: Human PrP (135 – 155) – P69 Control A – His Tag

SEQ ID NO. 21:

MRPIIHFGSDYEDRYYRENMHRVKTGERQHGIHIQGSDPGGVRTASGTTIKVSG
RQAQGILLENPAAELQFRNGSVTSSGQLSDDGIRRFLGTVTVKAGKLVADHATL
ANVGDTWDDDGIALYVAGEQAQASIADSTLQGAGGVQIERGANVTVQRSAIVD
GGLHIGALQSLQPEDLPPSRVVLRDTNVTAVPASGAPAAVSVLGASELTLDGGHI
TGGRAAGVAAMQGAVVHLQRATIRRGDALAGGAVPGGAVPGGAVPGGFGPG
GFGPVLDGWYGVDVSGSSVELAQSIVEAPELGAAIRVGRGARVTVPGGSLSAPH
GNVIETGGARRFAPQAAPLSITLQAGAHAQGKALLYRVLPEPVKLTLTGGADA
QGDIVATELPSIPGTSIGPLDVALASQARWTGATRAVDSLSIDNATWVMTDNSNV
GALRLASDGSVDFQQPAEAGRFKVLTVNTLAGSGLFRMNVFADLGLSDKLVVM
QDASGQHRLWVRNSGSEPASANTLLLVQTPLGSAATFTLANKDGKVDIGTYRY
RLAANGNGQWSLVGAKAPPGGHHHHHH

FIGURE 4A

PRION CHIMERAS USING P69 CONTROL A AND MOUSE PrP (135 – 155)

P69A/Mo135-155 Chimera No. 1: Mouse PrP (135 - 155) – P69 Control A – His Tag

SEQ ID NO. 22:
MRPMIHFGNDWEDRYYRENMYRDWNNQSIVKTGERQHGIHIQGSDPGGVRTA
SGTTIKVSGRQAQGILLENPAAELQFRNGSVTSSGQLSDDGIRRFLGTVTVKAGK
LVADHATLANVGDTWDDDGIALYVAGEQAQASIADSTLQGAGGVQIERGANVT
VQRSAIVDGGLHIGALQSLQPEDLPPSRVVLRDTNVTAVPASGAPAAVSVLGASE
LTLDGGHITGGRAAGVAAMQGAVVHLQRATIRRGDALAGGAVPGGAVPGGAV
PGGFGPGGFGPVLDGWYGVDVSGSSVELAQSIVEAPELGAAIRVGRGARVTVPG
GSLSAPHGNVIETGGARRFAPQAAPLSITLQAGAHAQGKALLYRVLPEPVKLTL
TGGADAQGDIVATELPSIPGTSIGPLDVALASQARWTGATRAVDSLSIDNATWV
MTDNSNVGALRLASDGSVDFQQPAEAGRFKVLTVNTLAGSGLFRMNVFADLGL
SDKLVVMQDASGQHRLWVRNSGSEPASANTLLLVQTPLGSAATFTLANKDGKV
DIGTYRYRLAANGNGQWSLVGAKAPPGGHHHHHH

P69A/Mo135-155 Chimera No. 2: Mouse PrP (135 - 155) – P69 Control A — His Tag

SEQ ID NO. 23:
MRPMIHFGNDWEDRYYRENMYRWNNQSIVKTGERQHGIHIQGSDPGGVRTAS
GTTIKVSGRQAQGILLENPAAELQFRNGSVTSSGQLSDDGIRRFLGTVTVKAGK
LVADHATLANVGDTWDDDGIALYVAGEQAQASIADSTLQGAGGVQIERGANVT
VQRSAIVDGGLHIGALQSLQPEDLPPSRVVLRDTNVTAVPASGAPAAVSVLGASE
LTLDGGHITGGRAAGVAAMQGAVVHLQRATIRRGDALAGGAVPGGAVPGGAV
PGGFGPGGFGPVLDGWYGVDVSGSSVELAQSIVEAPELGAAIRVGRGARVTVPG
GSLSAPHGNVIETGGARRFAPQAAPLSITLQAGAHAQGKALLYRVLPEPVKLTL
TGGADAQGDIVATELPSIPGTSIGPLDVALASQARWTGATRAVDSLSIDNATWV
MTDNSNVGALRLASDGSVDFQQPAEAGRFKVLTVNTLAGSGLFRMNVFADLGL
SDKLVVMQDASGQHRLWVRNSGSEPASANTLLLVQTPLGSAATFTLANKDGKV
DIGTYRYRLAANGNGQWSLVGAKAPPGGHHHHHH

FIGURE 4B

PRION CHIMERAS USING P69 CONTROL A AND MOUSE PrP (135 – 155)

P69A/Mo135-155 Chimera No. 3: Mouse PrP (135 - 155) – P69 Control A – His Tag

SEQ ID NO. 24:
MRPMIHFGNDWEDRYYRENMYRNNQSIVKTGERQHGIHIQGSDPGGVRTASGT
TIKVSGRQAQGILLENPAAELQFRNGSVTSSGQLSDDGIRRFLGTVTVKAGKLV
ADHATLANVGDTWDDDGIALYVAGEQAQASIADSTLQGAGGVQIERGANVTVQ
RSAIVDGGLHIGALQSLQPEDLPPSRVVLRDTNVTAVPASGAPAAVSVLGASELT
LDGGHITGGRAAGVAAMQGAVVHLQRATIRRGDALAGGAVPGGAVPGGAVPG
GFGPGGFGPVLDGWYGVDVSGSSVELAQSIVEAPELGAAIRVGRGARVTVPGGS
LSAPHGNVIETGGARRFAPQAAPLSITLQAGAHAQGKALLYRVLPEPVKLTLTG
GADAQGDIVATELPSIPGTSIGPLDVALASQARWTGATRAVDSLSIDNATWVMT
DNSNVGALRLASDGSVDFQQPAEAGRFKVLTVNTLAGSGLFRMNVFADLGLSD
KLVVMQDASGQHRLWVRNSGSEPASANTLLLVQTPLGSAATFTLANKDGKVDI
GTYRYRLAANGNGQWSLVGAKAPPGGHHHHHH

P69A/Mo135-155 Chimera No. 4: Mouse PrP (135 - 155) – P69 Control A – His Tag

SEQ ID NO. 25:
MRPMIHFGNDWEDRYYRENMYRNQSIVKTGERQHGIHIQGSDPGGVRTASGTT
IKVSGRQAQGILLENPAAELQFRNGSVTSSGQLSDDGIRRFLGTVTVKAGKLVA
DHATLANVGDTWDDDGIALYVAGEQAQASIADSTLQGAGGVQIERGANVTVQR
SAIVDGGLHIGALQSLQPEDLPPSRVVLRDTNVTAVPASGAPAAVSVLGASELTL
DGGHITGGRAAGVAAMQGAVVHLQRATIRRGDALAGGAVPGGAVPGGAVPGG
FGPGGFGPVLDGWYGVDVSGSSVELAQSIVEAPELGAAIRVGRGARVTVPGGSL
SAPHGNVIETGGARRFAPQAAPLSITLQAGAHAQGKALLYRVLPEPVKLTLTGG
ADAQGDIVATELPSIPGTSIGPLDVALASQARWTGATRAVDSLSIDNATWVMTD
NSNVGALRLASDGSVDFQQPAEAGRFKVLTVNTLAGSGLFRMNVFADLGLSDK
LVVMQDASGQHRLWVRNSGSEPASANTLLLVQTPLGSAATFTLANKDGKVDIG
TYRYRLAANGNGQWSLVGAKAPPGGHHHHHH

FIGURE 4C

PRION CHIMERAS USING P69 CONTROL A AND MOUSE PrP (135 – 155)

P69A/Mo135-155 Chimera No. 5: Mouse PrP (135 - 155) – P69 Control A – His Tag

SEQ ID NO. 26:
MRPMIHFGNDWEDRYYRENMYRQSIVKTGERQHGIHIQGSDPGGVRTASGTTI
KVSGRQAQGILLENPAAELQFRNGSVTSSGQLSDDGIRRFLGTVTVKAGKLVAD
HATLANVGDTWDDDGIALYVAGEQAQASIADSTLQGAGGVQIERGANVTVQRS
AIVDGGLHIGALQSLQPEDLPPSRVVLRDTNVTAVPASGAPAAVSVLGASELTLD
GGHITGGRAAGVAAMQGAVVHLQRATIRRGDALAGGAVPGGAVPGGAVPGGF
GPGGFGPVLDGWYGVDVSGSSVELAQSIVEAPELGAAIRVGRGARVTVPGGSLS
APHGNVIETGGARRFAPQAAPLSITLQAGAHAQGKALLYRVLPEPVKLTLTGGA
DAQGDIVATELPSIPGTSIGPLDVALASQARWTGATRAVDSLSIDNATWVMTDNS
NVGALRLASDGSVDFQQPAEAGRFKVLTVNTLAGSGLFRMNVFADLGLSDKLV
VMQDASGQHRLWVRNSGSEPASANTLLLVQTPLGSAATFTLANKDGKVDIGTY
RYRLAANGNGQWSLVGAKAPPGGHHHHHH

P69A/Mo135-155 Chimera No. 6: Mouse PrP (135 - 155) – P69 Control A – His Tag

SEQ ID NO. 27:
MRPMIHFGNDWEDRYYRENMYRSIVKTGERQHGIHIQGSDPGGVRTASGTTIK
VSGRQAQGILLENPAAELQFRNGSVTSSGQLSDDGIRRFLGTVTVKAGKLVADH
ATLANVGDTWDDDGIALYVAGEQAQASIADSTLQGAGGVQIERGANVTVQRSAI
VDGGLHIGALQSLQPEDLPPSRVVLRDTNVTAVPASGAPAAVSVLGASELTLDG
GHITGGRAAGVAAMQGAVVHLQRATIRRGDALAGGAVPGGAVPGGAVPGGFG
PGGFGPVLDGWYGVDVSGSSVELAQSIVEAPELGAAIRVGRGARVTVPGGSLSA
PHGNVIETGGARRFAPQAAPLSITLQAGAHAQGKALLYRVLPEPVKLTLTGGAD
AQGDIVATELPSIPGTSIGPLDVALASQARWTGATRAVDSLSIDNATWVMTDNSN
VGALRLASDGSVDFQQPAEAGRFKVLTVNTLAGSGLFRMNVFADLGLSDKLVV
MQDASGQHRLWVRNSGSEPASANTLLLVQTPLGSAATFTLANKDGKVDIGTYR
YRLAANGNGQWSLVGAKAPPGGHHHHHH

FIGURE 4D

PRION CHIMERAS USING P69 CONTROL A AND MOUSE PrP (135 – 155)

P69A/Mo135-155 Chimera No. 7: Mouse PrP (135 - 155) – P69 Control A – His Tag

SEQ ID NO. 28:
MRPMIHFGNDWEDRYYRENMYRIVKTGERQHGIHIQGSDPGGVRTASGTTIKV
SGRQAQGILLENPAAELQFRNGSVTSSGQLSDDGIRRFLGTVTVKAGKLVADHA
TLANVGDTWDDDGIALYVAGEQAQASIADSTLQGAGGVQIERGANVTVQRSAIV
DGGLHIGALQSLQPEDLPPSRVVLRDTNVTAVPASGAPAAVSVLGASELTLDGG
HITGGRAAGVAAMQGAVVHLQRATIRRGDALAGGAVPGGAVPGGAVPGGFGP
GGFGPVLDGWYGVDVSGSSVELAQSIVEAPELGAAIRVGRGARVTVPGGSLSAP
HGNVIETGGARRFAPQAAPLSITLQAGAHAQGKALLYRVLPEPVKLTLTGGAD
AQGDIVATELPSIPGTSIGPLDVALASQARWTGATRAVDSLSIDNATWVMTDNSN
VGALRLASDGSVDFQQPAEAGRFKVLTVNTLAGSGLFRMNVFADLGLSDKLVV
MQDASGQHRLWVRNSGSEPASANTLLLVQTPLGSAATFTLANKDGKVDIGTYR
YRLAANGNGQWSLVGAKAPPGGHHHHHH

P69A/Mo135-155 Chimera No. 8: Mouse PrP (135 - 155) – P69 Control A – His Tag

SEQ ID NO. 29:
MRPMIHFGNDWEDRYYRENMYRVKTGERQHGIHIQGSDPGGVRTASGTTIKVS
GRQAQGILLENPAAELQFRNGSVTSSGQLSDDGIRRFLGTVTVKAGKLVADHAT
LANVGDTWDDDGIALYVAGEQAQASIADSTLQGAGGVQIERGANVTVQRSAIV
DGGLHIGALQSLQPEDLPPSRVVLRDTNVTAVPASGAPAAVSVLGASELTLDGG
HITGGRAAGVAAMQGAVVHLQRATIRRGDALAGGAVPGGAVPGGAVPGGFGP
GGFGPVLDGWYGVDVSGSSVELAQSIVEAPELGAAIRVGRGARVTVPGGSLSAP
HGNVIETGGARRFAPQAAPLSITLQAGAHAQGKALLYRVLPEPVKLTLTGGAD
AQGDIVATELPSIPGTSIGPLDVALASQARWTGATRAVDSLSIDNATWVMTDNSN
VGALRLASDGSVDFQQPAEAGRFKVLTVNTLAGSGLFRMNVFADLGLSDKLVV
MQDASGQHRLWVRNSGSEPASANTLLLVQTPLGSAATFTLANKDGKVDIGTYR
YRLAANGNGQWSLVGAKAPPGGHHHHHH

FIGURE 5A

PRION CHIMERAS USING P69 CONTROL B AND HUMAN PrP (135 – 155)

P69B/Hu135-155 Chimera No. 1: His Tag - P69 Control B – HuPrP (135-155)

SEQ ID NO. 30:
MHHHHHHGGMNMSLSRIVKAAPLRRTTLAMALGALGAAPAAHADWNNQSIV
KTGERQHGIHIQGSDPGGVRTASGTTIKVSGRQAQGILLENPAAELQFRNGSVTS
SGQLSDDGIRRFLGTVTVKAGKLVADHATLANVGDTWDDDGIALYVAGEQAQA
SIADSTLQGAGGVQIERGANVTVQRSAIVDGGLHIGALQSLQPEDLPPSRVVLRD
TNVTAVPASGAPAAVSVLGASELTLDGGHITGGRAAGVAAMQGAVVHLQRATI
RRGDALAGGAVPGGAVPGGAVPGGFGPGGFGPVLDGWYGVDVSGSSVELAQSI
VEAPELGAAIRVGRGARVTVPGGSLSAPHGNVIETGGARRFAPQAAPLSITLQA
GAHAQGKALLYRVLPEPVKLTLTGGADAQGDIVATELPSIPGTSIGPLDVALASQ
ARWTGATRAVDSLSIDNATWVMTDNSNVGALRLASDGSVDFQQPAEAGRFKVL
TVNTLARPIIHFGSDYEDRYYRENMHR

P69B/Hu135-155 Chimera No. 2: His Tag - P69 Control B – HuPrP (135-155)

SEQ ID NO. 31:
MHHHHHHGGMNMSLSRIVKAAPLRRTTLAMALGALGAAPAAHADWNNQSIV
KTGERQHGIHIQGSDPGGVRTASGTTIKVSGRQAQGILLENPAAELQFRNGSVTS
SGQLSDDGIRRFLGTVTVKAGKLVADHATLANVGDTWDDDGIALYVAGEQAQA
SIADSTLQGAGGVQIERGANVTVQRSAIVDGGLHIGALQSLQPEDLPPSRVVLRD
TNVTAVPASGAPAAVSVLGASELTLDGGHITGGRAAGVAAMQGAVVHLQRATI
RRGDALAGGAVPGGAVPGGAVPGGFGPGGFGPVLDGWYGVDVSGSSVELAQSI
VEAPELGAAIRVGRGARVTVPGGSLSAPHGNVIETGGARRFAPQAAPLSITLQA
GAHAQGKALLYRVLPEPVKLTLTGGADAQGDIVATELPSIPGTSIGPLDVALASQ
ARWTGATRAVDSLSIDNATWVMTDNSNVGALRLASDGSVDFQQPAEAGRFKVL
TVNTLRPIIHFGSDYEDRYYRENMHR

FIGURE 5B

PRION CHIMERAS USING P69 CONTROL B AND HUMAN PrP (135 – 155)

P69B/Hu135-155 Chimera No. 3: His Tag - P69 Control B – HuPrP (135-155)

SEQ ID NO. 32:
MHHHHHHGGMNMSLSRIVKAAPLRRTTLAMALGALGAAPAAHADWNNQSIV
KTGERQHGIHIQGSDPGGVRTASGTTIKVSGRQAQGILLENPAAELQFRNGSVTS
SGQLSDDGIRRFLGTVTVKAGKLVADHATLANVGDTWDDDGIALYVAGEQAQA
SIADSTLQGAGGVQIERGANVTVQRSAIVDGGLHIGALQSLQPEDLPPSRVVLRD
TNVTAVPASGAPAAVSVLGASELTLDGGHITGGRAAGVAAMQGAVVHLQRATI
RRGDALAGGAVPGGAVPGGAVPGGFGPGGFGPVLDGWYGVDVSGSSVELAQSI
VEAPELGAAIRVGRGARVTVPGGSLSAPHGNVIETGGARRFAPQAAPLSITLQA
GAHAQGKALLYRVLPEPVKLTLTGGADAQGDIVATELPSIPGTSIGPLDVALASQ
ARWTGATRAVDSLSIDNATWVMTDNSNVGALRLASDGSVDFQQPAEAGRFKVL
TVNTRPIIHFGSDYEDRYYRENMHR

P69B/Hu135-155 Chimera No. 4: His Tag - P69 Control B – HuPrP (135-155)

SEQ ID NO. 33:
MHHHHHHGGMNMSLSRIVKAAPLRRTTLAMALGALGAAPAAHADWNNQSIV
KTGERQHGIHIQGSDPGGVRTASGTTIKVSGRQAQGILLENPAAELQFRNGSVTS
SGQLSDDGIRRFLGTVTVKAGKLVADHATLANVGDTWDDDGIALYVAGEQAQA
SIADSTLQGAGGVQIERGANVTVQRSAIVDGGLHIGALQSLQPEDLPPSRVVLRD
TNVTAVPASGAPAAVSVLGASELTLDGGHITGGRAAGVAAMQGAVVHLQRATI
RRGDALAGGAVPGGAVPGGAVPGGFGPGGFGPVLDGWYGVDVSGSSVELAQSI
VEAPELGAAIRVGRGARVTVPGGSLSAPHGNVIETGGARRFAPQAAPLSITLQA
GAHAQGKALLYRVLPEPVKLTLTGGADAQGDIVATELPSIPGTSIGPLDVALASQ
ARWTGATRAVDSLSIDNATWVMTDNSNVGALRLASDGSVDFQQPAEAGRFKVL
TVNRPIIHFGSDYEDRYYRENMHR

FIGURE 5C

PRION CHIMERAS USING P69 CONTROL B AND HUMAN PrP (135 – 155)

P69B/Hu135-155 Chimera No. 5: His Tag - P69 Control B – HuPrP (135-155)

SEQ ID NO. 34:
MHHHHHHGGMNMSLSRIVKAAPLRRTTLAMALGALGAAPAAHADWNNQSIV
KTGERQHGIHIQGSDPGGVRTASGTTIKVSGRQAQGILLENPAAELQFRNGSVTS
SGQLSDDGIRRFLGTVTVKAGKLVADHATLANVGDTWDDDGIALYVAGEQAQA
SIADSTLQGAGGVQIERGANVTVQRSAIVDGGLHIGALQSLQPEDLPPSRVVLRD
TNVTAVPASGAPAAVSVLGASELTLDGGHITGGRAAGVAAMQGAVVHLQRATI
RRGDALAGGAVPGGAVPGGAVPGGFGPGGFGPVLDGWYGVDVSGSSVELAQSI
VEAPELGAAIRVGRGARVTVPGGSLSAPHGNVIETGGARRFAPQAAPLSITLQA
GAHAQGKALLYRVLPEPVKLTLTGGADAQGDIVATELPSIPGTSIGPLDVALASQ
ARWTGATRAVDSLSIDNATWVMTDNSNVGALRLASDGSVDFQQPAEAGRFKVL
TVRPIIHFGSDYEDRYYRENMHR

P69B/Hu135-155 Chimera No. 6: His Tag - P69 Control B – HuPrP (135-155)

SEQ ID NO. 35:
MHHHHHHGGMNMSLSRIVKAAPLRRTTLAMALGALGAAPAAHADWNNQSIV
KTGERQHGIHIQGSDPGGVRTASGTTIKVSGRQAQGILLENPAAELQFRNGSVTS
SGQLSDDGIRRFLGTVTVKAGKLVADHATLANVGDTWDDDGIALYVAGEQAQA
SIADSTLQGAGGVQIERGANVTVQRSAIVDGGLHIGALQSLQPEDLPPSRVVLRD
TNVTAVPASGAPAAVSVLGASELTLDGGHITGGRAAGVAAMQGAVVHLQRATI
RRGDALAGGAVPGGAVPGGAVPGGFGPGGFGPVLDGWYGVDVSGSSVELAQSI
VEAPELGAAIRVGRGARVTVPGGSLSAPHGNVIETGGARRFAPQAAPLSITLQA
GAHAQGKALLYRVLPEPVKLTLTGGADAQGDIVATELPSIPGTSIGPLDVALASQ
ARWTGATRAVDSLSIDNATWVMTDNSNVGALRLASDGSVDFQQPAEAGRFKVL
TRPIIHFGSDYEDRYYRENMHR

FIGURE 5D

PRION CHIMERAS USING P69 CONTROL B AND HUMAN PrP (135 – 155)

P69B/Hu135-155 Chimera No. 7: His Tag - P69 Control B – HuPrP (135-155)

SEQ ID NO. 36:
MHHHHHHGGMNMSLSRIVKAAPLRRTTLAMALGALGAAPAAHADWNNQSIV
KTGERQHGIHIQGSDPGGVRTASGTTIKVSGRQAQGILLENPAAELQFRNGSVTS
SGQLSDDGIRRFLGTVTVKAGKLVADHATLANVGDTWDDDGIALYVAGEQAQA
SIADSTLQGAGGVQIERGANVTVQRSAIVDGGLHIGALQSLQPEDLPPSRVVLRD
TNVTAVPASGAPAAVSVLGASELTLDGGHITGGRAAGVAAMQGAVVHLQRATI
RRGDALAGGAVPGGAVPGGAVPGGFGPGGFGPVLDGWYGVDVSGSSVELAQSI
VEAPELGAAIRVGRGARVTVPGGSLSAPHGNVIETGGARRFAPQAAPLSITLQA
GAHAQGKALLYRVLPEPVKLTLTGGADAQGDIVATELPSIPGTSIGPLDVALASQ
ARWTGATRAVDSLSIDNATWVMTDNSNVGALRLASDGSVDFQQPAEAGRFKVL
RPIIHFGSDYEDRYYRENMHR

P69B/Hu135-155 Chimera No. 8: His Tag - P69 Control B – HuPrP (135-155)

SEQ ID NO. 37:
MHHHHHHGGMNMSLSRIVKAAPLRRTTLAMALGALGAAPAAHADWNNQSIV
KTGERQHGIHIQGSDPGGVRTASGTTIKVSGRQAQGILLENPAAELQFRNGSVTS
SGQLSDDGIRRFLGTVTVKAGKLVADHATLANVGDTWDDDGIALYVAGEQAQA
SIADSTLQGAGGVQIERGANVTVQRSAIVDGGLHIGALQSLQPEDLPPSRVVLRD
TNVTAVPASGAPAAVSVLGASELTLDGGHITGGRAAGVAAMQGAVVHLQRATI
RRGDALAGGAVPGGAVPGGAVPGGFGPGGFGPVLDGWYGVDVSGSSVELAQSI
VEAPELGAAIRVGRGARVTVPGGSLSAPHGNVIETGGARRFAPQAAPLSITLQA
GAHAQGKALLYRVLPEPVKLTLTGGADAQGDIVATELPSIPGTSIGPLDVALASQ
ARWTGATRAVDSLSIDNATWVMTDNSNVGALRLASDGSVDFQQPAEAGRFKVR
PIIHFGSDYEDRYYRENMHR

FIGURE 6A

PRION CHIMERAS USING P69 CONTROL B AND MOUSE PrP (135-155)

P69B/Mo135-155 Chimera No. 1: His Tag – P69 Control B – Mouse PrP (135 – 155):

SEQ ID NO. 38:
MHHHHHHGGMNMSLSRIVKAAPLRRTTLAMALGALGAAPAAHADWNNQSIV
KTGERQHGIHIQGSDPGGVRTASGTTIKVSGRQAQGILLENPAAELQFRNGSVTS
SGQLSDDGIRRFLGTVTVKAGKLVADHATLANVGDTWDDDGIALYVAGEQAQA
SIADSTLQGAGGVQIERGANVTVQRSAIVDGGLHIGALQSLQPEDLPPSRVVLRD
TNVTAVPASGAPAAVSVLGASELTLDGGHITGGRAAGVAAMQGAVVHLQRATI
RRGDALAGGAVPGGAVPGGAVPGGFGPGGFGPVLDGWYGVDVSGSSVELAQSI
VEAPELGAAIRVGRGARVTVPGGSLSAPHGNVIETGGARRFAPQAAPLSITLQA
GAHAQGKALLYRVLPEPVKLTLTGGADAQGDIVATELPSIPGTSIGPLDVALASQ
ARWTGATRAVDSLSIDNATWVMTDNSNVGALRLASDGSVDFQQPAEAGRFKVL
TVNTLARPMIHFGNDWEDRYYRENMYR

P69B/Mo135-155 Chimera No. 2: His Tag – P69 Control B – Mouse PrP (135 – 155):

SEQ ID NO. 39:
MHHHHHHGGMNMSLSRIVKAAPLRRTTLAMALGALGAAPAAHADWNNQSIV
KTGERQHGIHIQGSDPGGVRTASGTTIKVSGRQAQGILLENPAAELQFRNGSVTS
SGQLSDDGIRRFLGTVTVKAGKLVADHATLANVGDTWDDDGIALYVAGEQAQA
SIADSTLQGAGGVQIERGANVTVQRSAIVDGGLHIGALQSLQPEDLPPSRVVLRD
TNVTAVPASGAPAAVSVLGASELTLDGGHITGGRAAGVAAMQGAVVHLQRATI
RRGDALAGGAVPGGAVPGGAVPGGFGPGGFGPVLDGWYGVDVSGSSVELAQSI
VEAPELGAAIRVGRGARVTVPGGSLSAPHGNVIETGGARRFAPQAAPLSITLQA
GAHAQGKALLYRVLPEPVKLTLTGGADAQGDIVATELPSIPGTSIGPLDVALASQ
ARWTGATRAVDSLSIDNATWVMTDNSNVGALRLASDGSVDFQQPAEAGRFKVL
TVNTLRPMIHFGNDWEDRYYRENMYR

FIGURE 6B

PRION CHIMERAS USING P69 CONTROL B AND MOUSE PrP (135-155)

P69B/Mo135-155 Chimera No. 3: His Tag – P69 Control B – Mouse PrP (135 – 155):

SEQ ID NO. 40:
MHHHHHHGGMNMSLSRIVKAAPLRRTTLAMALGALGAAPAAHADWNNQSIV KTGERQHGIHIQGSDPGGVRTASGTTIKVSGRQAQGILLENPAAELQFRNGSVTS SGQLSDDGIRRFLGTVTVKAGKLVADHATLANVGDTWDDDGIALYVAGEQAQA SIADSTLQGAGGVQIERGANVTVQRSAIVDGGLHIGALQSLQPEDLPPSRVVLRD TNVTAVPASGAPAAVSVLGASELTLDGGHITGGRAAGVAAMQGAVVHLQRATI RRGDALAGGAVPGGAVPGGAVPGGFGPGGFGPVLDGWYGVDVSGSSVELAQSI VEAPELGAAIRVGRGARVTVPGGSLSAPHGNVIETGGARRFAPQAAPLSITLQA GAHAQGKALLYRVLPEPVKLTLTGGADAQGDIVATELPSIPGTSIGPLDVALASQ ARWTGATRAVDSLSIDNATWVMTDNSNVGALRLASDGSVDFQQPAEAGRFKVL TVNTRPMIHFGNDWEDRYYRENMYR

P69B/Mo135-155 Chimera No. 4: His Tag – P69 Control B – Mouse PrP (135 – 155):

SEQ ID NO. 41:
MHHHHHHGGMNMSLSRIVKAAPLRRTTLAMALGALGAAPAAHADWNNQSIV KTGERQHGIHIQGSDPGGVRTASGTTIKVSGRQAQGILLENPAAELQFRNGSVTS SGQLSDDGIRRFLGTVTVKAGKLVADHATLANVGDTWDDDGIALYVAGEQAQA SIADSTLQGAGGVQIERGANVTVQRSAIVDGGLHIGALQSLQPEDLPPSRVVLRD TNVTAVPASGAPAAVSVLGASELTLDGGHITGGRAAGVAAMQGAVVHLQRATI RRGDALAGGAVPGGAVPGGAVPGGFGPGGFGPVLDGWYGVDVSGSSVELAQSI VEAPELGAAIRVGRGARVTVPGGSLSAPHGNVIETGGARRFAPQAAPLSITLQA GAHAQGKALLYRVLPEPVKLTLTGGADAQGDIVATELPSIPGTSIGPLDVALASQ ARWTGATRAVDSLSIDNATWVMTDNSNVGALRLASDGSVDFQQPAEAGRFKVL TVNRPMIHFGNDWEDRYYRENMYR

FIGURE 6C

PRION CHIMERAS USING P69 CONTROL B AND MOUSE PrP (135-155)

P69B/Mo135-155 Chimera No. 5: His Tag – P69 Control B – Mouse PrP (135 – 155):

SEQ ID NO. 42:
MHHHHHHGGMNMSLSRIVKAAPLRRTTLAMALGALGAAPAAHADWNNQSIV
KTGERQHGIHIQGSDPGGVRTASGTTIKVSGRQAQGILLENPAAELQFRNGSVTS
SGQLSDDGIRRFLGTVTVKAGKLVADHATLANVGDTWDDDGIALYVAGEQAQA
SIADSTLQGAGGVQIERGANVTVQRSAIVDGGLHIGALQSLQPEDLPPSRVVLRD
TNVTAVPASGAPAAVSVLGASELTLDGGHITGGRAAGVAAMQGAVVHLQRATI
RRGDALAGGAVPGGAVPGGAVPGGFGPGGFGPVLDGWYGVDVSGSSVELAQSI
VEAPELGAAIRVGRGARVTVPGGSLSAPHGNVIETGGARRFAPQAAPLSITLQA
GAHAQGKALLYRVLPEPVKLTLTGGADAQGDIVATELPSIPGTSIGPLDVALASQ
ARWTGATRAVDSLSIDNATWVMTDNSNVGALRLASDGSVDFQQPAEAGRFKVL
TVRPMIHFGNDWEDRYYRENMYR

P69B/Mo135-155 Chimera No. 6: His Tag – P69 Control B – Mouse PrP (135 – 155):

SEQ ID NO. 43:
MHHHHHHGGMNMSLSRIVKAAPLRRTTLAMALGALGAAPAAHADWNNQSIV
KTGERQHGIHIQGSDPGGVRTASGTTIKVSGRQAQGILLENPAAELQFRNGSVTS
SGQLSDDGIRRFLGTVTVKAGKLVADHATLANVGDTWDDDGIALYVAGEQAQA
SIADSTLQGAGGVQIERGANVTVQRSAIVDGGLHIGALQSLQPEDLPPSRVVLRD
TNVTAVPASGAPAAVSVLGASELTLDGGHITGGRAAGVAAMQGAVVHLQRATI
RRGDALAGGAVPGGAVPGGAVPGGFGPGGFGPVLDGWYGVDVSGSSVELAQSI
VEAPELGAAIRVGRGARVTVPGGSLSAPHGNVIETGGARRFAPQAAPLSITLQA
GAHAQGKALLYRVLPEPVKLTLTGGADAQGDIVATELPSIPGTSIGPLDVALASQ
ARWTGATRAVDSLSIDNATWVMTDNSNVGALRLASDGSVDFQQPAEAGRFKVL
TRPMIHFGNDWEDRYYRENMYR

FIGURE 6D

PRION CHIMERAS USING P69 CONTROL B AND MOUSE PrP (135-155)

P69B/Mo135-155 Chimera No. 7: His Tag – P69 Control B – Mouse PrP (135 – 155):

SEQ ID NO. 44:
MHHHHHHGGMNMSLSRIVKAAPLRRTTLAMALGALGAAPAAHADWNNQSIV KTGERQHGIHIQGSDPGGVRTASGTTIKVSGRQAQGILLENPAAELQFRNGSVTS SGQLSDDGIRRFLGTVTVKAGKLVADHATLANVGDTWDDDGIALYVAGEQAQA SIADSTLQGAGGVQIERGANVTVQRSAIVDGGLHIGALQSLQPEDLPPSRVVLRD TNVTAVPASGAPAAVSVLGASELTLDGGHITGGRAAGVAAMQGAVVHLQRATI RRGDALAGGAVPGGAVPGGAVPGGFGPGGFGPVLDGWYGVDVSGSSVELAQSI VEAPELGAAIRVGRGARVTVPGGSLSAPHGNVIETGGARRFAPQAAPLSITLQA GAHAQGKALLYRVLPEPVKLTLTGGADAQGDIVATELPSIPGTSIGPLDVALASQ ARWTGATRAVDSLSIDNATWVMTDNSNVGALRLASDGSVDFQQPAEAGRFKVL RPMIHFGNDWEDRYYRENMYR

P69B/Mo135-155 Chimera No. 8: His Tag – P69 Control B – Mouse PrP (135 – 155):

SEQ ID NO. 45:
MHHHHHHGGMNMSLSRIVKAAPLRRTTLAMALGALGAAPAAHADWNNQSIV KTGERQHGIHIQGSDPGGVRTASGTTIKVSGRQAQGILLENPAAELQFRNGSVTS SGQLSDDGIRRFLGTVTVKAGKLVADHATLANVGDTWDDDGIALYVAGEQAQA SIADSTLQGAGGVQIERGANVTVQRSAIVDGGLHIGALQSLQPEDLPPSRVVLRD TNVTAVPASGAPAAVSVLGASELTLDGGHITGGRAAGVAAMQGAVVHLQRATI RRGDALAGGAVPGGAVPGGAVPGGFGPGGFGPVLDGWYGVDVSGSSVELAQSI VEAPELGAAIRVGRGARVTVPGGSLSAPHGNVIETGGARRFAPQAAPLSITLQA GAHAQGKALLYRVLPEPVKLTLTGGADAQGDIVATELPSIPGTSIGPLDVALASQ ARWTGATRAVDSLSIDNATWVMTDNSNVGALRLASDGSVDFQQPAEAGRFKVR PMIHFGNDWEDRYYRENMYR

FIGURE 7

GCA AMINO ACID SEQUENCES

Full Length Amino Acid Sequence of GCA

SEQ ID NO. 4: **MMFNKQIFTILILSLSLALAGSGCISEGAED NVAQEITVDEFSNIRENPVTPWNPEPSAPVIDPTAYIDPQA SVIGEVTIGANVMVSPMASIRSDEGMPIFVGDRSNVQDGV VLHALETINEEGEPIEDNIVEVDGKEYAVYIGNNVSLAHQS QVHGPAAVGDDTFIGMQAFVFKSKVGNNCVLEPRSAAIG VTIPDGRYIPAGMVVTSQAEADKLPEVTDDYAYSHTNEAV VYVNVHLAEGYKETS**

Full Length Amino Acid Sequence of GCA - leader sequence removed

SEQ ID NO:183: **QEITVDEFSNIRENPVTPWNPEPSAPVIDP TAYIDPQASVIGEVTIGANVMVSPMASIRSDEGMPIFVGDR SNVQDGVVLHALETINEEGEPIEDNIVEVDGKEYAVYIGN NVSLAHQSQVHGPAAVGDDTFIGMQAFVFKSKVGNNCVL EPRSAAIGVTIPDGRYIPAGMVVTSQAEADKLPEVTDDYA YSHTNEAVVYVNVHLAEGYKETS**

Amino Acid Sequence of GCA Control A:

SEQ ID NO. 10: TAYIDPQASVIGEVTIGANVMVSPMASIRSDEG MPIFVGDRSNVQDGVVLHALETINEEGEPIEDNIVEVDGKEY AVYIGNNVSLAHQSQVHGPAAVGDDTFIGMQAFVFKSKVGN NCVLEPRSAAIGVTIPDGRYIPAGMVVTSQAEADKLPEVTDD YAYSHTNEAVVYVNVHLAEGYKETS

Amino Acid Sequence of GCA Control B:

SEQ ID NO. 11: MMFNKQIFTILILSLSLALAGSGCISEGAEDNVA QEITVDEFSNIRENPVTPWNPEPSAPVIDPTAYIDPQASVIGE VTIGANVMVSPMASIRSDEGMPIFVGDRSNVQDGVVLHALE TINEEGEPIEDNIVEVDGKEYAVYIGNNVSLAHQSQVHGPAA VGDDTFIGMQAFVFKSKVGNNCVLEPRSAAIGVTIPDGRYIP AGMVVT

Amino Acid Sequence of GCA Control B (leader sequence removed):

SEQ ID NO:184: QEITVDEFSNIRENPVTPWNPEPSAPVIDPTAYI DPQASVIGEVTIGANVMVSPMASIRSDEGMPIFVGDRSNVQD GVVLHALETINEEGEPIEDNIVEVDGKEYAVYIGNNVSLAHQ SQVHGPAAVGDDTFIGMQAFVFKSKVGNNCVLEPRSAAIGV TIPDGRYIPAGMVVT

FIGURE 8A

PRION CHIMERAS USING GCA CONTROL A AND HUMAN PrP (135-155)

GCAA/HuPrP135-155 Chimera No. 1: HuPrP (135-155) – GCA Control A – His Tag:

SEQ ID NO. 46:
MRPIIHFGSDYEDRYYRENMHRTAYIDPQASVIGEVTIGANVMVSPMASIRSDEG MPIFVGDRSNVQDGVVLHALETINEEGEPIEDNIVEVDGKEYAVYIGNNVSLAHQ SQVHGPAAVGDDTFIGMQAFVFKSKVGNNCVLEPRSAAIGVTIPDGRYIPAGMV VTSQAEADKLPEVTDDYAYSHTNEAVVYVNVHLAEGYKETSGGHHHHHH

GCAA/HuPrP135-155 Chimera No. 2: HuPrP (135-155) – GCA Control A – His Tag:

SEQ ID NO. 47:
MRPIIHFGSDYEDRYYRENMHRAYIDPQASVIGEVTIGANVMVSPMASIRSDEG MPIFVGDRSNVQDGVVLHALETINEEGEPIEDNIVEVDGKEYAVYIGNNVSLAHQ SQVHGPAAVGDDTFIGMQAFVFKSKVGNNCVLEPRSAAIGVTIPDGRYIPAGMV VTSQAEADKLPEVTDDYAYSHTNEAVVYVNVHLAEGYKETSGGHHHHHH

GCAA/HuPrP135-155 Chimera No. 3: HuPrP (135-155) – GCA Control A – His Tag:

SEQ ID NO. 48:
MRPIIHFGSDYEDRYYRENMHRYIDPQASVIGEVTIGANVMVSPMASIRSDEGMP IFVGDRSNVQDGVVLHALETINEEGEPIEDNIVEVDGKEYAVYIGNNVSLAHQSQ VHGPAAVGDDTFIGMQAFVFKSKVGNNCVLEPRSAAIGVTIPDGRYIPAGMVVT SQAEADKLPEVTDDYAYSHTNEAVVYVNVHLAEGYKETSGGHHHHHH

FIGURE 8B

PRION CHIMERAS USING GCA CONTROL A AND HUMAN PrP (135-155)

GCAA/HuPrP135-155 Chimera No. 4: HuPrP (135-155) – GCA Control A – His Tag:

SEQ ID NO. 49:
MRPIIHFGSDYEDRYYRENMHRIDPQASVIGEVTIGANVMVSPMASIRSDEGMPI FVGDRSNVQDGVVLHALETINEEGEPIEDNIVEVDGKEYAVYIGNNVSLAHQSQ VHGPAAVGDDTFIGMQAFVFKSKVGNNCVLEPRSAAIGVTIPDGRYIPAGMVVT SQAEADKLPEVTDDYAYSHTNEAVVYVNVHLAEGYKETSGGHHHHHH

GCAA/HuPrP135-155 Chimera No. 5: HuPrP (135-155) – GCA Control A – His Tag:

SEQ ID NO. 50:
MRPIIHFGSDYEDRYYRENMHRDPQASVIGEVTIGANVMVSPMASIRSDEGMPIF VGDRSNVQDGVVLHALETINEEGEPIEDNIVEVDGKEYAVYIGNNVSLAHQSQV HGPAAVGDDTFIGMQAFVFKSKVGNNCVLEPRSAAIGVTIPDGRYIPAGMVVTS QAEADKLPEVTDDYAYSHTNEAVVYVNVHLAEGYKETSGGHHHHHH

GCAA/HuPrP135-155 Chimera No. 6: HuPrP (135-155) – GCA Control A – His Tag:

SEQ ID NO. 51:
MRPIIHFGSDYEDRYYRENMHRPQASVIGEVTIGANVMVSPMASIRSDEGMPIFV GDRSNVQDGVVLHALETINEEGEPIEDNIVEVDGKEYAVYIGNNVSLAHQSQVH GPAAVGDDTFIGMQAFVFKSKVGNNCVLEPRSAAIGVTIPDGRYIPAGMVVTSQ AEADKLPEVTDDYAYSHTNEAVVYVNVHLAEGYKETSGGHHHHHH

FIGURE 9A

PRION CHIMERAS USING GCA CONTROL A AND MOUSE PrP (135 – 155)

GCAA/MoPrP135-155 Chimera No. 1: Mo PrP (135-155) – GCA Control A – His Tag:

SEQ ID NO. 52:
MRPMIHFGNDWEDRYYRENMYRTAYIDPQASVIGEVTIGANVMVSPMASIRSDE GMPIFVGDRSNVQDGVVLHALETINEEGEPIEDNIVEVDGKEYAVYIGNNVSLAH QSQVHGPAAVGDDTFIGMQAFVFKSKVGNNCVLEPRSAAIGVTIPDGRYIPAGM VVTSQAEADKLPEVTDDYAYSHTNEAVVYVNVHLAEGYKETSGGHHHHHH

GCAA/MoPrP135-155 Chimera No. 2: Mo PrP (135-155) – GCA Control A – His Tag:

SEQ ID NO. 53:
MRPMIHFGNDWEDRYYRENMYRAYIDPQASVIGEVTIGANVMVSPMASIRSDE GMPIFVGDRSNVQDGVVLHALETINEEGEPIEDNIVEVDGKEYAVYIGNNVSLAH QSQVHGPAAVGDDTFIGMQAFVFKSKVGNNCVLEPRSAAIGVTIPDGRYIPAGM VVTSQAEADKLPEVTDDYAYSHTNEAVVYVNVHLAEGYKETSGGHHHHHH

GCAA/MoPrP135-155 Chimera No. 3: Mo PrP (135-155) – GCA Control A – His Tag:

SEQ ID NO. 54:
MRPMIHFGNDWEDRYYRENMYRYIDPQASVIGEVTIGANVMVSPMASIRSDEG MPIFVGDRSNVQDGVVLHALETINEEGEPIEDNIVEVDGKEYAVYIGNNVSLAHQ SQVHGPAAVGDDTFIGMQAFVFKSKVGNNCVLEPRSAAIGVTIPDGRYIPAGMV VTSQAEADKLPEVTDDYAYSHTNEAVVYVNVHLAEGYKETSGGHHHHHH

FIGURE 9B

PRION CHIMERAS USING GCA CONTROL A AND MOUSE PrP (135 – 155)

GCAA/MoPrP135-155 Chimera No. 4: Mo PrP (135-155) – GCA Control A – His Tag:

SEQ ID NO. 55:
MRPMIHFGNDWEDRYYRENMYRIDPQASVIGEVTIGANVMVSPMASIRSDEGM PIFVGDRSNVQDGVVLHALETINEEGEPIEDNIVEVDGKEYAVYIGNNVSLAHQS QVHGPAAVGDDTFIGMQAFVFKSKVGNNCVLEPRSAAIGVTIPDGRYIPAGMVV TSQAEADKLPEVTDDYAYSHTNEAVVYVNVHLAEGYKETSGGHHHHHH

GCAA/MoPrP135-155 Chimera No. 5: Mo PrP (135-155) – GCA Control A – His Tag:

SEQ ID NO. 56:
MRPMIHFGNDWEDRYYRENMYRDPQASVIGEVTIGANVMVSPMASIRSDEGMP IFVGDRSNVQDGVVLHALETINEEGEPIEDNIVEVDGKEYAVYIGNNVSLAHQSQ VHGPAAVGDDTFIGMQAFVFKSKVGNNCVLEPRSAAIGVTIPDGRYIPAGMVVT SQAEADKLPEVTDDYAYSHTNEAVVYVNVHLAEGYKETSGGHHHHHH

GCAA/MoPrP135-155 Chimera No. 6: Mo PrP (135-155) – GCA Control A – His Tag:

SEQ ID NO. 57:
MRPMIHFGNDWEDRYYRENMYRPQASVIGEVTIGANVMVSPMASIRSDEGMPI FVGDRSNVQDGVVLHALETINEEGEPIEDNIVEVDGKEYAVYIGNNVSLAHQSQ VHGPAAVGDDTFIGMQAFVFKSKVGNNCVLEPRSAAIGVTIPDGRYIPAGMVVT SQAEADKLPEVTDDYAYSHTNEAVVYVNVHLAEGYKETSGGHHHHHH

FIGURE 10A

PRION CHIMERAS USING GCA CONTROL B AND HUMAN PrP (135-155)

GCAB/HuPrP135-155 Chimera No. 1: His Tag – GCA Control B – Hu PrP (135 – 155):

SEQ ID NO. 58:

MHHHHHHGGMMFNKQIFTILILSLSLALAGSGCISEGAEDNVAQEITVDEFSNI
RENPVTPWNPEPSAPVIDPTAYIDPQASVIGEVTIGANVMVSPMASIRSDEGMPIFVGD
RSNVQDGVVLHALETINEEGEPIEDNIVEVDGKEYAVYIGNNVSLAHQSQVHGPAAV
GDDTFIGMQAFVFKSKVGNNCVLEPRSAAIGVTIPDGRYIPAGMVVTRPIIHFGSDYE
DRYYRENMHR

GCAB/HuPrP135-155 Chimera No. 2: His Tag – GCA Control B – Hu PrP (135 – 155):

SEQ ID NO. 59:

MHHHHHHGGMMFNKQIFTILILSLSLALAGSGCISEGAEDNVAQEITVDEFSNI
RENPVTPWNPEPSAPVIDPTAYIDPQASVIGEVTIGANVMVSPMASIRSDEGMPIFVGD
RSNVQDGVVLHALETINEEGEPIEDNIVEVDGKEYAVYIGNNVSLAHQSQVHGPAAV
GDDTFIGMQAFVFKSKVGNNCVLEPRSAAIGVTIPDGRYIPAGMVVRPIIHFGSDYED
RYYRENMHR

GCAB/HuPrP135-155 Chimera No. 3: His Tag – GCA Control B – Hu PrP (135 – 155):

SEQ ID NO. 60:

MHHHHHHGGMMFNKQIFTILILSLSLALAGSGCISEGAEDNVAQEITVDEFSNI
RENPVTPWNPEPSAPVIDPTAYIDPQASVIGEVTIGANVMVSPMASIRSDEGMPIFVGD
RSNVQDGVVLHALETINEEGEPIEDNIVEVDGKEYAVYIGNNVSLAHQSQVHGPAAV
GDDTFIGMQAFVFKSKVGNNCVLEPRSAAIGVTIPDGRYIPAGMVRPIIHFGSDYEDR
YYRENMHR

FIGURE 10B

PRION CHIMERAS USING GCA CONTROL B AND HUMAN PrP (135-155)

GCAB/HuPrP135-155 Chimera No. 4: His Tag – GCA Control B – Hu PrP (135 – 155):

SEQ ID NO. 61:

MHHHHHHGGMMFNKQIFTILILSLSLALAGSGCISEGAEDNVAQEITVDEFSNI
RENPVTPWNPEPSAPVIDPTAYIDPQASVIGEVTIGANVMVSPMASIRSDEGMPIFVGD
RSNVQDGVVLHALETINEEGEPIEDNIVEVDGKEYAVYIGNNVSLAHQSQVHGPAAV
GDDTFIGMQAFVFKSKVGNNCVLEPRSAAIGVTIPDGRYIPAGMRPIIHFGSDYEDRY
YRENMHR

GCAB/HuPrP135-155 Chimera No. 5: His Tag – GCA Control B – Hu PrP (135 – 155):

SEQ ID NO. 62:

MHHHHHHGGMMFNKQIFTILILSLSLALAGSGCISEGAEDNVAQEITVDEFSNI
RENPVTPWNPEPSAPVIDPTAYIDPQASVIGEVTIGANVMVSPMASIRSDEGMPIFVGD
RSNVQDGVVLHALETINEEGEPIEDNIVEVDGKEYAVYIGNNVSLAHQSQVHGPAAV
GDDTFIGMQAFVFKSKVGNNCVLEPRSAAIGVTIPDGRYIPAGRPIIHFGSDYEDRYY
RENMHR

GCAB/HuPrP135-155 Chimera No. 6: His Tag – GCA Control B – Hu PrP (135 – 155):

SEQ ID NO. 63:

MHHHHHHGGMMFNKQIFTILILSLSLALAGSGCISEGAEDNVAQEITVDEFSNI
RENPVTPWNPEPSAPVIDPTAYIDPQASVIGEVTIGANVMVSPMASIRSDEGMPIFVGD
RSNVQDGVVLHALETINEEGEPIEDNIVEVDGKEYAVYIGNNVSLAHQSQVHGPAAV
GDDTFIGMQAFVFKSKVGNNCVLEPRSAAIGVTIPDGRYIPARPIIHFGSDYEDRYYRE
NMHR

FIGURE 11A

PRION CHIMERAS USING GCA CONTROL B AND MOUSE PrP (135 – 155)

GCAB/MoPrP135-155 Chimera No. 1: His Tag – GCA Control B – Mo PrP (135 – 155):

SEQ ID NO. 64:

MHHHHHHGGMMFNKQIFTILILSLSLALAGSGCISEGAEDNVAQEITVDEFSNI
RENPVTPWNPEPSAPVIDPTAYIDPQASVIGEVTIGANVMVSPMASIRSDEGMPIFVGD
RSNVQDGVVLHALETINEEGEPIEDNIVEVDGKEYAVYIGNNVSLAHQSQVHGPAAV
GDDTFIGMQAFVFKSKVGNNCVLEPRSAAIGVTIPDGRYIPAGMVVTMRPMIHFGND
WEDRYYRENMYR

GCAB/MoPrP135-155 Chimera No. 2: His Tag – GCA Control B – Mo PrP (135 – 155):

SEQ ID NO. 65:

MHHHHHHGGMMFNKQIFTILILSLSLALAGSGCISEGAEDNVAQEITVDEFSNI
RENPVTPWNPEPSAPVIDPTAYIDPQASVIGEVTIGANVMVSPMASIRSDEGMPIFVGD
RSNVQDGVVLHALETINEEGEPIEDNIVEVDGKEYAVYIGNNVSLAHQSQVHGPAAV
GDDTFIGMQAFVFKSKVGNNCVLEPRSAAIGVTIPDGRYIPAGMVVTRPMIHFGNDW
EDRYYRENMYR

GCAB/MoPrP135-155 Chimera No. 3: His Tag – GCA Control B – Mo PrP (135 – 155):

SEQ ID NO. 66:

MHHHHHHGGMMFNKQIFTILILSLSLALAGSGCISEGAEDNVAQEITVDEFSNI
RENPVTPWNPEPSAPVIDPTAYIDPQASVIGEVTIGANVMVSPMASIRSDEGMPIFVGD
RSNVQDGVVLHALETINEEGEPIEDNIVEVDGKEYAVYIGNNVSLAHQSQVHGPAAV
GDDTFIGMQAFVFKSKVGNNCVLEPRSAAIGVTIPDGRYIPAGMVVRPMIHFGNDWE
DRYYRENMYR

FIGURE 11B

PRION CHIMERAS USING GCA CONTROL B AND MOUSE PrP (135 – 155)

GCAB/MoPrP135-155 Chimera No. 4: His Tag – GCA Control B – Mo PrP (135 – 155):

SEQ ID NO. 67:

MHHHHHHGGMMFNKQIFTILILSLSLALAGSGCISEGAEDNVAQEITVDEFSNI
RENPVTPWNPEPSAPVIDPTAYIDPQASVIGEVTIGANVMVSPMASIRSDEGMPIFVGD
RSNVQDGVVLHALETINEEGEPIEDNIVEVDGKEYAVYIGNNVSLAHQSQVHGPAAV
GDDTFIGMQAFVFKSKVGNNCVLEPRSAAIGVTIPDGRYIPAGMVRPMIHFGNDWED
RYYRENMYR

GCAB/MoPrP135-155 Chimera No. 5: His Tag – GCA Control B – Mo PrP (135 – 155):

SEQ ID NO. 68:

MHHHHHHGGMMFNKQIFTILILSLSLALAGSGCISEGAEDNVAQEITVDEFSNI
RENPVTPWNPEPSAPVIDPTAYIDPQASVIGEVTIGANVMVSPMASIRSDEGMPIFVGD
RSNVQDGVVLHALETINEEGEPIEDNIVEVDGKEYAVYIGNNVSLAHQSQVHGPAAV
GDDTFIGMQAFVFKSKVGNNCVLEPRSAAIGVTIPDGRYIPAGMRPMIHFGNDWEDR
YYRENMYR

GCAB/MoPrP135-155 Chimera No. 6: His Tag – GCA Control B – Mo PrP (135 – 155):

SEQ ID NO. 69:

MHHHHHHGGMMFNKQIFTILILSLSLALAGSGCISEGAEDNVAQEITVDEFSNI
RENPVTPWNPEPSAPVIDPTAYIDPQASVIGEVTIGANVMVSPMASIRSDEGMPIFVGD
RSNVQDGVVLHALETINEEGEPIEDNIVEVDGKEYAVYIGNNVSLAHQSQVHGPAAV
GDDTFIGMQAFVFKSKVGNNCVLEPRSAAIGVTIPDGRYIPAGRPMIHFGNDWEDRY
YRENMYR

FIGURE 12A

PRION CHIMERAS USING P69 CONTROL A AND HUMAN PrP (126 – 154)

P69/Hu126-154 Chimera No. 1: Human PrP (126 – 154) – P69 Control A – His Tag

SEQ ID NO. 70:
MGYMLGSAMSRPIIHFGSDYEDRYYRENMHDWNNQSIVKTGERQHGIHIQGSD
PGGVRTASGTTIKVSGRQAQGILLENPAAELQFRNGSVTSSGQLSDDGIRRFLGT
VTVKAGKLVADHATLANVGDTWDDDGIALYVAGEQAQASIADSTLQGAGGVQI
ERGANVTVQRSAIVDGGLHIGALQSLQPEDLPPSRVVLRDTNVTAVPASGAPAA
VSVLGASELTLDGGHITGGRAAGVAAMQGAVVHLQRATIRRGDALAGGAVPG
GAVPGGAVPGGFGPGGFGPVLDGWYGVDVSGSSVELAQSIVEAPELGAAIRVG
RGARVTVPGGSLSAPHGNVIETGGARRFAPQAAPLSITLQAGAHAQGKALLYRV
LPEPVKLTLTGGADAQGDIVATELPSIPGTSIGPLDVALASQARWTGATRAVDSL
SIDNATWVMTDNSNVGALRLASDGSVDFQQPAEAGRFKVLTVNTLAGSGLFRM
NVFADLGLSDKLVVMQDASGQHRLWVRNSGSEPASANTLLLVQTPLGSAATFT
LANKDGKVDIGTYRYRLAANGNGQWSLVGAKAPPGGHHHHHH

P69/Hu126-154 Chimera No. 2: Human PrP (126-154) – P69 Control A – His Tag

SEQ ID NO. 71:
MGYMLGSAMSRPIIHFGSDYEDRYYRENMHWNNQSIVKTGERQHGIHIQGSDP
GGVRTASGTTIKVSGRQAQGILLENPAAELQFRNGSVTSSGQLSDDGIRRFLGTV
TVKAGKLVADHATLANVGDTWDDDGIALYVAGEQAQASIADSTLQGAGGVQIE
RGANVTVQRSAIVDGGLHIGALQSLQPEDLPPSRVVLRDTNVTAVPASGAPAAV
SVLGASELTLDGGHITGGRAAGVAAMQGAVVHLQRATIRRGDALAGGAVPGG
AVPGGAVPGGFGPGGFGPVLDGWYGVDVSGSSVELAQSIVEAPELGAAIRVGR
GARVTVPGGSLSAPHGNVIETGGARRFAPQAAPLSITLQAGAHAQGKALLYRVL
PEPVKLTLTGGADAQGDIVATELPSIPGTSIGPLDVALASQARWTGATRAVDSLS
IDNATWVMTDNSNVGALRLASDGSVDFQQPAEAGRFKVLTVNTLAGSGLFRMN
VFADLGLSDKLVVMQDASGQHRLWVRNSGSEPASANTLLLVQTPLGSAATFTL
ANKDGKVDIGTYRYRLAANGNGQWSLVGAKAPPGGHHHHHH

FIGURE 12B

PRION CHIMERAS USING P69 CONTROL A AND HUMAN PrP (126 – 154)

P69/Hu126-154 Chimera No. 3: Human PrP (126-154) – P69 Control A – His Tag

SEQ ID NO. 72:
MGYMLGSAMSRPIIHFGSDYEDRYYRENMHNNQSIVKTGERQHGIHIQGSDPG
GVRTASGTTIKVSGRQAQGILLENPAAELQFRNGSVTSSGQLSDDGIRRFLGTVT
VKAGKLVADHATLANVGDTWDDDGIALYVAGEQAQASIADSTLQGAGGVQIER
GANVTVQRSAIVDGGLHIGALQSLQPEDLPPSRVVLRDTNVTAVPASGAPAAVS
VLGASELTLDGGHITGGRAAGVAAMQGAVVHLQRATIRRGDALAGGAVPGGA
VPGGAVPGGFGPGGFGPVLDGWYGVDVSGSSVELAQSIVEAPELGAAIRVGRG
ARVTVPGGSLSAPHGNVIETGGARRFAPQAAPLSITLQAGAHAQGKALLYRVLP
EPVKLTLTGGADAQGDIVATELPSIPGTSIGPLDVALASQARWTGATRAVDSLSI
DNATWVMTDNSNVGALRLASDGSVDFQQPAEAGRFKVLTVNTLAGSGLFRMN
VFADLGLSDKLVVMQDASGQHRLWVRNSGSEPASANTLLLVQTPLGSAATFTL
ANKDGKVDIGTYRYRLAANGNGQWSLVGAKAPPGGHHHHHH

P69/Hu126-154 Chimera No. 4: Human PrP (126-154) – P69 Control A – His Tag

SEQ ID NO. 73:
MGYMLGSAMSRPIIHFGSDYEDRYYRENMHNQSIVKTGERQHGIHIQGSDPGG
VRTASGTTIKVSGRQAQGILLENPAAELQFRNGSVTSSGQLSDDGIRRFLGTVTV
KAGKLVADHATLANVGDTWDDDGIALYVAGEQAQASIADSTLQGAGGVQIERG
ANVTVQRSAIVDGGLHIGALQSLQPEDLPPSRVVLRDTNVTAVPASGAPAAVSVL
GASELTLDGGHITGGRAAGVAAMQGAVVHLQRATIRRGDALAGGAVPGGAVP
GGAVPGGFGPGGFGPVLDGWYGVDVSGSSVELAQSIVEAPELGAAIRVGRGAR
VTVPGGSLSAPHGNVIETGGARRFAPQAAPLSITLQAGAHAQGKALLYRVLPEP
VKLTLTGGADAQGDIVATELPSIPGTSIGPLDVALASQARWTGATRAVDSLSIDN
ATWVMTDNSNVGALRLASDGSVDFQQPAEAGRFKVLTVNTLAGSGLFRMNVF
ADLGLSDKLVVMQDASGQHRLWVRNSGSEPASANTLLLVQTPLGSAATFTLAN
KDGKVDIGTYRYRLAANGNGQWSLVGAKAPPGGHHHHHH

FIGURE 12C

PRION CHIMERAS USING P69 CONTROL A AND HUMAN PrP (126 – 154)

P69/Hu126-154 Chimera No. 5: Human PrP (126-154) – P69 Control A – His Tag

SEQ ID NO. 74:
MGYMLGSAMSRPIIHFGSDYEDRYYRENMHQSIVKTGERQHGIHIQGSDPGGV
RTASGTTIKVSGRQAQGILLENPAAELQFRNGSVTSSGQLSDDGIRRFLGTVTVK
AGKLVADHATLANVGDTWDDDGIALYVAGEQAQASIADSTLQGAGGVQIERGA
NVTVQRSAIVDGGLHIGALQSLQPEDLPPSRVVLRDTNVTAVPASGAPAAVSVLG
ASELTLDGGHITGGRAAGVAAMQGAVVHLQRATIRRGDALAGGAVPGGAVPG
GAVPGGFGPGGFGPVLDGWYGVDVSGSSVELAQSIVEAPELGAAIRVGRGARV
TVPGGSLSAPHGNVIETGGARRFAPQAAPLSITLQAGAHAQGKALLYRVLPEPV
KLTLTGGADAQGDIVATELPSIPGTSIGPLDVALASQARWTGATRAVDSLSIDNA
TWVMTDNSNVGALRLASDGSVDFQQPAEAGRFKVLTVNTLAGSGLFRMNVFA
DLGLSDKLVVMQDASGQHRLWVRNSGSEPASANTLLLVQTPLGSAATFTLANK
DGKVDIGTYRYRLAANGNGQWSLVGAKAPPGGHHHHHH

P69/Hu126-154 Chimera No. 6: Human PrP (126-154) – P69 Control A – His Tag

SEQ ID NO. 75:
MGYMLGSAMSRPIIHFGSDYEDRYYRENMHSIVKTGERQHGIHIQGSDPGGVRT
ASGTTIKVSGRQAQGILLENPAAELQFRNGSVTSSGQLSDDGIRRFLGTVTVKAG
KLVADHATLANVGDTWDDDGIALYVAGEQAQASIADSTLQGAGGVQIERGANV
TVQRSAIVDGGLHIGALQSLQPEDLPPSRVVLRDTNVTAVPASGAPAAVSVLGAS
ELTLDGGHITGGRAAGVAAMQGAVVHLQRATIRRGDALAGGAVPGGAVPGGA
VPGGFGPGGFGPVLDGWYGVDVSGSSVELAQSIVEAPELGAAIRVGRGARVTVP
GGSLSAPHGNVIETGGARRFAPQAAPLSITLQAGAHAQGKALLYRVLPEPVKLT
LTGGADAQGDIVATELPSIPGTSIGPLDVALASQARWTGATRAVDSLSIDNATWV
MTDNSNVGALRLASDGSVDFQQPAEAGRFKVLTVNTLAGSGLFRMNVFADLGL
SDKLVVMQDASGQHRLWVRNSGSEPASANTLLLVQTPLGSAATFTLANKDGKV
DIGTYRYRLAANGNGQWSLVGAKAPPGGHHHHHH

FIGURE 12D

PRION CHIMERAS USING P69 CONTROL A AND HUMAN PrP (126 – 154)

P69/Hu126-154 Chimera No. 7: Human PrP (126-154) – P69 Control A – His Tag

SEQ ID NO. 76:
MGYMLGSAMSRPIIHFGSDYEDRYYRENMHIVKTGERQHGIHIQGSDPGGVRT
ASGTTIKVSGRQAQGILLENPAAELQFRNGSVTSSGQLSDDGIRRFLGTVTVKAG
KLVADHATLANVGDTWDDDGIALYVAGEQAQASIADSTLQGAGGVQIERGANV
TVQRSAIVDGGLHIGALQSLQPEDLPPSRVVLRDTNVTAVPASGAPAAVSVLGAS
ELTLDGGHITGGRAAGVAAMQGAVVHLQRATIRRGDALAGGAVPGGAVPGGA
VPGGFGPGGFGPVLDGWYGVDVSGSSVELAQSIVEAPELGAAIRVGRGARVTVP
GGSLSAPHGNVIETGGARRFAPQAAPLSITLQAGAHAQGKALLYRVLPEPVKLT
LTGGADAQGDIVATELPSIPGTSIGPLDVALASQARWTGATRAVDSLSIDNATWV
MTDNSNVGALRLASDGSVDFQQPAEAGRFKVLTVNTLAGSGLFRMNVFADLGL
SDKLVVMQDASGQHRLWVRNSGSEPASANTLLLVQTPLGSAATFTLANKDGKV
DIGTYRYRLAANGNGQWSLVGAKAPPGGHHHHHH

P69/Hu126-154 Chimera No. 8: Human PrP (126-154) – P69 Control A – His Tag

SEQ ID NO. 77:
MGYMLGSAMSRPIIHFGSDYEDRYYRENMHVKTGERQHGIHIQGSDPGGVRTA
SGTTIKVSGRQAQGILLENPAAELQFRNGSVTSSGQLSDDGIRRFLGTVTVKAGK
LVADHATLANVGDTWDDDGIALYVAGEQAQASIADSTLQGAGGVQIERGANVT
VQRSAIVDGGLHIGALQSLQPEDLPPSRVVLRDTNVTAVPASGAPAAVSVLGASE
LTLDGGHITGGRAAGVAAMQGAVVHLQRATIRRGDALAGGAVPGGAVPGGAV
PGGFGPGGFGPVLDGWYGVDVSGSSVELAQSIVEAPELGAAIRVGRGARVTVPG
GSLSAPHGNVIETGGARRFAPQAAPLSITLQAGAHAQGKALLYRVLPEPVKLTL
TGGADAQGDIVATELPSIPGTSIGPLDVALASQARWTGATRAVDSLSIDNATWV
MTDNSNVGALRLASDGSVDFQQPAEAGRFKVLTVNTLAGSGLFRMNVFADLGL
SDKLVVMQDASGQHRLWVRNSGSEPASANTLLLVQTPLGSAATFTLANKDGKV
DIGTYRYRLAANGNGQWSLVGAKAPPGGHHHHHH

FIGURE 13A

PRION CHIMERAS USING P69 CONTROL A AND MOUSE PrP (126 – 154)

P69A/Mo126-154 Chimera No. 1: Mouse PrP (126 - 154) – P69 Control A – His Tag

SEQ ID NO. 78:
MGYMLGSAMSRPMIHFGNDWEDRYYRENMYDWNNQSIVKTGERQHGIHIQGS
DPGGVRTASGTTIKVSGRQAQGILLENPAAELQFRNGSVTSSGQLSDDGIRRFLG
TVTVKAGKLVADHATLANVGDTWDDDGIALYVAGEQAQASIADSTLQGAGGV
QIERGANVTVQRSAIVDGGLHIGALQSLQPEDLPPSRVVLRDTNVTAVPASGAPA
AVSVLGASELTLDGGHITGGRAAGVAAMQGAVVHLQRATIRRGDALAGGAVP
GGAVPGGAVPGGFGPGGFGPVLDGWYGVDVSGSSVELAQSIVEAPELGAAIRV
GRGARVTVPGGSLSAPHGNVIETGGARRFAPQAAPLSITLQAGAHAQGKALLYR
VLPEPVKLTLTGGADAQGDIVATELPSIPGTSIGPLDVALASQARWTGATRAVDS
LSIDNATWVMTDNSNVGALRLASDGSVDFQQPAEAGRFKVLTVNTLAGSGLFR
MNVFADLGLSDKLVVMQDASGQHRLWVRNSGSEPASANTLLLVQTPLGSAATF
TLANKDGKVDIGTYRYRLAANGNGQWSLVGAKAPPGGHHHHHH

P69A/Mo126-154 Chimera No. 2: Mouse PrP (126-154) – P69 Control A – His Tag

SEQ ID NO. 79:
MGYMLGSAMSRPMIHFGNDWEDRYYRENMYWNNQSIVKTGERQHGIHIQGSD
PGGVRTASGTTIKVSGRQAQGILLENPAAELQFRNGSVTSSGQLSDDGIRRFLGT
VTVKAGKLVADHATLANVGDTWDDDGIALYVAGEQAQASIADSTLQGAGGVQI
ERGANVTVQRSAIVDGGLHIGALQSLQPEDLPPSRVVLRDTNVTAVPASGAPAA
VSVLGASELTLDGGHITGGRAAGVAAMQGAVVHLQRATIRRGDALAGGAVPG
GAVPGGAVPGGFGPGGFGPVLDGWYGVDVSGSSVELAQSIVEAPELGAAIRVG
RGARVTVPGGSLSAPHGNVIETGGARRFAPQAAPLSITLQAGAHAQGKALLYRV
LPEPVKLTLTGGADAQGDIVATELPSIPGTSIGPLDVALASQARWTGATRAVDSL
SIDNATWVMTDNSNVGALRLASDGSVDFQQPAEAGRFKVLTVNTLAGSGLFRM
NVFADLGLSDKLVVMQDASGQHRLWVRNSGSEPASANTLLLVQTPLGSAATFT
LANKDGKVDIGTYRYRLAANGNGQWSLVGAKAPPGGHHHHHH

FIGURE 13B

PRION CHIMERAS USING P69 CONTROL A AND MOUSE PrP (126 – 154)

P69A/Mo126-154 Chimera No. 3: Mouse PrP (126-154) – P69 Control A – His Tag

SEQ ID NO. 80:
MGYMLGSAMSRPMIHFGNDWEDRYYRENMYNNQSIVKTGERQHGIHIQGSDP
GGVRTASGTTIKVSGRQAQGILLENPAAELQFRNGSVTSSGQLSDDGIRRFLGTV
TVKAGKLVADHATLANVGDTWDDDGIALYVAGEQAQASIADSTLQGAGGVQIE
RGANVTVQRSAIVDGGLHIGALQSLQPEDLPPSRVVLRDTNVTAVPASGAPAAV
SVLGASELTLDGGHITGGRAAGVAAMQGAVVHLQRATIRRGDALAGGAVPGG
AVPGGAVPGGFGPGGFGPVLDGWYGVDVSGSSVELAQSIVEAPELGAAIRVGR
GARVTVPGGSLSAPHGNVIETGGARRFAPQAAPLSITLQAGAHAQGKALLYRVL
PEPVKLTLTGGADAQGDIVATELPSIPGTSIGPLDVALASQARWTGATRAVDSLS
IDNATWVMTDNSNVGALRLASDGSVDFQQPAEAGRFKVLTVNTLAGSGLFRMN
VFADLGLSDKLVVMQDASGQHRLWVRNSGSEPASANTLLLVQTPLGSAATFTL
ANKDGKVDIGTYRYRLAANGNGQWSLVGAKAPPGGHHHHHH

P69A/Mo126-154 Chimera No. 4: Mouse PrP (126-154) – P69 Control A – His Tag

SEQ ID NO. 81:
MGYMLGSAMSRPMIHFGNDWEDRYYRENMYNQSIVKTGERQHGIHIQGSDPG
GVRTASGTTIKVSGRQAQGILLENPAAELQFRNGSVTSSGQLSDDGIRRFLGTVT
VKAGKLVADHATLANVGDTWDDDGIALYVAGEQAQASIADSTLQGAGGVQIER
GANVTVQRSAIVDGGLHIGALQSLQPEDLPPSRVVLRDTNVTAVPASGAPAAVS
VLGASELTLDGGHITGGRAAGVAAMQGAVVHLQRATIRRGDALAGGAVPGGA
VPGGAVPGGFGPGGFGPVLDGWYGVDVSGSSVELAQSIVEAPELGAAIRVGRG
ARVTVPGGSLSAPHGNVIETGGARRFAPQAAPLSITLQAGAHAQGKALLYRVLP
EPVKLTLTGGADAQGDIVATELPSIPGTSIGPLDVALASQARWTGATRAVDSLSI
DNATWVMTDNSNVGALRLASDGSVDFQQPAEAGRFKVLTVNTLAGSGLFRMN
VFADLGLSDKLVVMQDASGQHRLWVRNSGSEPASANTLLLVQTPLGSAATFTL
ANKDGKVDIGTYRYRLAANGNGQWSLVGAKAPPGGHHHHHH

FIGURE 13C

PRION CHIMERAS USING P69 CONTROL A AND MOUSE PrP (126 – 154)

P69A/Mo126-154 Chimera No. 5: Mouse PrP (126-154) – P69 Control A – His Tag

SEQ ID NO. 82:
MGYMLGSAMSRPMIHFGNDWEDRYYRENMYQSIVKTGERQHGIHIQGSDPGG
VRTASGTTIKVSGRQAQGILLENPAAELQFRNGSVTSSGQLSDDGIRRFLGTVTV
KAGKLVADHATLANVGDTWDDDGIALYVAGEQAQASIADSTLQGAGGVQIERG
ANVTVQRSAIVDGGLHIGALQSLQPEDLPPSRVVLRDTNVTAVPASGAPAAVSVL
GASELTLDGGHITGGRAAGVAAMQGAVVHLQRATIRRGDALAGGAVPGGAVP
GGAVPGGFGPGGFGPVLDGWYGVDVSGSSVELAQSIVEAPELGAAIRVGRGAR
VTVPGGSLSAPHGNVIETGGARRFAPQAAPLSITLQAGAHAQGKALLYRVLPEP
VKLTLTGGADAQGDIVATELPSIPGTSIGPLDVALASQARWTGATRAVDSLSIDN
ATWVMTDNSNVGALRLASDGSVDFQQPAEAGRFKVLTVNTLAGSGLFRMNVF
ADLGLSDKLVVMQDASGQHRLWVRNSGSEPASANTLLLVQTPLGSAATFTLAN
KDGKVDIGTYRYRLAANGNGQWSLVGAKAPPGGHHHHHH

P69A/Mo126-154 Chimera No. 6: Mouse PrP (126-154) – P69 Control A – His Tag

SEQ ID NO. 83:
MGYMLGSAMSRPMIHFGNDWEDRYYRENMYSIVKTGERQHGIHIQGSDPGGV
RTASGTTIKVSGRQAQGILLENPAAELQFRNGSVTSSGQLSDDGIRRFLGTVTVK
AGKLVADHATLANVGDTWDDDGIALYVAGEQAQASIADSTLQGAGGVQIERGA
NVTVQRSAIVDGGLHIGALQSLQPEDLPPSRVVLRDTNVTAVPASGAPAAVSVLG
ASELTLDGGHITGGRAAGVAAMQGAVVHLQRATIRRGDALAGGAVPGGAVPG
GAVPGGFGPGGFGPVLDGWYGVDVSGSSVELAQSIVEAPELGAAIRVGRGARV
TVPGGSLSAPHGNVIETGGARRFAPQAAPLSITLQAGAHAQGKALLYRVLPEPV
KLTLTGGADAQGDIVATELPSIPGTSIGPLDVALASQARWTGATRAVDSLSIDNA
TWVMTDNSNVGALRLASDGSVDFQQPAEAGRFKVLTVNTLAGSGLFRMNVFA
DLGLSDKLVVMQDASGQHRLWVRNSGSEPASANTLLLVQTPLGSAATFTLANK
DGKVDIGTYRYRLAANGNGQWSLVGAKAPPGGHHHHHH

FIGURE 13D

PRION CHIMERAS USING P69 CONTROL A AND MOUSE PrP (126 – 154)

P69A/Mo126-154 Chimera No. 7: Mouse PrP (126-154) – P69 Control A – His Tag

SEQ ID NO. 84:
MGYMLGSAMSRPMIHFGNDWEDRYYRENMYIVKTGERQHGIHIQGSDPGGVR
TASGTTIKVSGRQAQGILLENPAAELQFRNGSVTSSGQLSDDGIRRFLGTVTVKA
GKLVADHATLANVGDTWDDDGIALYVAGEQAQASIADSTLQGAGGVQIERGAN
VTVQRSAIVDGGLHIGALQSLQPEDLPPSRVVLRDTNVTAVPASGAPAAVSVLGA
SELTLDGGHITGGRAAGVAAMQGAVVHLQRATIRRGDALAGGAVPGGAVPGG
AVPGGFGPGGFGPVLDGWYGVDVSGSSVELAQSIVEAPELGAAIRVGRGARVTV
PGGSLSAPHGNVIETGGARRFAPQAAPLSITLQAGAHAQGKALLYRVLPEPVKL
TLTGGADAQGDIVATELPSIPGTSIGPLDVALASQARWTGATRAVDSLSIDNATW
VMTDNSNVGALRLASDGSVDFQQPAEAGRFKVLTVNTLAGSGLFRMNVFADLG
LSDKLVVMQDASGQHRLWVRNSGSEPASANTLLLVQTPLGSAATFTLANKDGK
VDIGTYRYRLAANGNGQWSLVGAKAPPGGHHHHHH

P69A/Mo126-154 Chimera No. 8: Mouse PrP (126-154) – P69 Control A – His Tag

SEQ ID NO. 85:
MGYMLGSAMSRPMIHFGNDWEDRYYRENMYVKTGERQHGIHIQGSDPGGVRT
ASGTTIKVSGRQAQGILLENPAAELQFRNGSVTSSGQLSDDGIRRFLGTVTVKAG
KLVADHATLANVGDTWDDDGIALYVAGEQAQASIADSTLQGAGGVQIERGANV
TVQRSAIVDGGLHIGALQSLQPEDLPPSRVVLRDTNVTAVPASGAPAAVSVLGAS
ELTLDGGHITGGRAAGVAAMQGAVVHLQRATIRRGDALAGGAVPGGAVPGGA
VPGGFGPGGFGPVLDGWYGVDVSGSSVELAQSIVEAPELGAAIRVGRGARVTVP
GGSLSAPHGNVIETGGARRFAPQAAPLSITLQAGAHAQGKALLYRVLPEPVKLT
LTGGADAQGDIVATELPSIPGTSIGPLDVALASQARWTGATRAVDSLSIDNATWV
MTDNSNVGALRLASDGSVDFQQPAEAGRFKVLTVNTLAGSGLFRMNVFADLGL
SDKLVVMQDASGQHRLWVRNSGSEPASANTLLLVQTPLGSAATFTLANKDGKV
DIGTYRYRLAANGNGQWSLVGAKAPPGGHHHHHH

FIGURE 14A

PRION CHIMERAS USING P69 CONTROL B AND HUMAN PrP (126 – 154)

P69B/Hu126-154 Chimera No. 1: His Tag - P69 Control B – HuPrP (126-154)

SEQ ID NO. 86:
MHHHHHHGGMNMSLSRIVKAAPLRRTTLAMALGALGAAPAAHADWNNQSIV
KTGERQHGIHIQGSDPGGVRTASGTTIKVSGRQAQGILLENPAAELQFRNGSVTS
SGQLSDDGIRRFLGTVTVKAGKLVADHATLANVGDTWDDDGIALYVAGEQAQA
SIADSTLQGAGGVQIERGANVTVQRSAIVDGGLHIGALQSLQPEDLPPSRVVLRD
TNVTAVPASGAPAAVSVLGASELTLDGGHITGGRAAGVAAMQGAVVHLQRATI
RRGDALAGGAVPGGAVPGGAVPGGFGPGGFGPVLDGWYGVDVSGSSVELAQSI
VEAPELGAAIRVGRGARVTVPGGSLSAPHGNVIETGGARRFAPQAAPLSITLQA
GAHAQGKALLYRVLPEPVKLTLTGGADAQGDIVATELPSIPGTSIGPLDVALASQ
ARWTGATRAVDSLSIDNATWVMTDNSNVGALRLASDGSVDFQQPAEAGRFKVL
TVNTLAGYMLGSAMSRPIIHFGSDYEDRYYRENMH

P69B/Hu126-154 Chimera No. 2: His Tag - P69 Control B – HuPrP (126-154)

SEQ ID NO. 87:
MHHHHHHGGMNMSLSRIVKAAPLRRTTLAMALGALGAAPAAHADWNNQSIV
KTGERQHGIHIQGSDPGGVRTASGTTIKVSGRQAQGILLENPAAELQFRNGSVTS
SGQLSDDGIRRFLGTVTVKAGKLVADHATLANVGDTWDDDGIALYVAGEQAQA
SIADSTLQGAGGVQIERGANVTVQRSAIVDGGLHIGALQSLQPEDLPPSRVVLRD
TNVTAVPASGAPAAVSVLGASELTLDGGHITGGRAAGVAAMQGAVVHLQRATI
RRGDALAGGAVPGGAVPGGAVPGGFGPGGFGPVLDGWYGVDVSGSSVELAQSI
VEAPELGAAIRVGRGARVTVPGGSLSAPHGNVIETGGARRFAPQAAPLSITLQA
GAHAQGKALLYRVLPEPVKLTLTGGADAQGDIVATELPSIPGTSIGPLDVALASQ
ARWTGATRAVDSLSIDNATWVMTDNSNVGALRLASDGSVDFQQPAEAGRFKVL
TVNTLGYMLGSAMSRPIIHFGSDYEDRYYRENMH

FIGURE 14B

PRION CHIMERAS USING P69 CONTROL B AND HUMAN PrP (126 – 154)

P69B/Hu126-154 Chimera No. 3: His Tag - P69 Control B – HuPrP (126-154)

SEQ ID NO. 88:
MHHHHHHGGMNMSLSRIVKAAPLRRTTLAMALGALGAAPAAHADWNNQSIV
KTGERQHGIHIQGSDPGGVRTASGTTIKVSGRQAQGILLENPAAELQFRNGSVTS
SGQLSDDGIRRFLGTVTVKAGKLVADHATLANVGDTWDDDGIALYVAGEQAQA
SIADSTLQGAGGVQIERGANVTVQRSAIVDGGLHIGALQSLQPEDLPPSRVVLRD
TNVTAVPASGAPAAVSVLGASELTLDGGHITGGRAAGVAAMQGAVVHLQRATI
RRGDALAGGAVPGGAVPGGAVPGGFGPGGFGPVLDGWYGVDVSGSSVELAQSI
VEAPELGAAIRVGRGARVTVPGGSLSAPHGNVIETGGARRFAPQAAPLSITLQA
GAHAQGKALLYRVLPEPVKLTLTGGADAQGDIVATELPSIPGTSIGPLDVALASQ
ARWTGATRAVDSLSIDNATWVMTDNSNVGALRLASDGSVDFQQPAEAGRFKVL
TVNTGYMLGSAMSRPIIHFGSDYEDRYYRENMH

P69B/Hu126-154 Chimera No. 4: His Tag - P69 Control B – HuPrP (126-154)

SEQ ID NO. 89:
MHHHHHHGGMNMSLSRIVKAAPLRRTTLAMALGALGAAPAAHADWNNQSIV
KTGERQHGIHIQGSDPGGVRTASGTTIKVSGRQAQGILLENPAAELQFRNGSVTS
SGQLSDDGIRRFLGTVTVKAGKLVADHATLANVGDTWDDDGIALYVAGEQAQA
SIADSTLQGAGGVQIERGANVTVQRSAIVDGGLHIGALQSLQPEDLPPSRVVLRD
TNVTAVPASGAPAAVSVLGASELTLDGGHITGGRAAGVAAMQGAVVHLQRATI
RRGDALAGGAVPGGAVPGGAVPGGFGPGGFGPVLDGWYGVDVSGSSVELAQSI
VEAPELGAAIRVGRGARVTVPGGSLSAPHGNVIETGGARRFAPQAAPLSITLQA
GAHAQGKALLYRVLPEPVKLTLTGGADAQGDIVATELPSIPGTSIGPLDVALASQ
ARWTGATRAVDSLSIDNATWVMTDNSNVGALRLASDGSVDFQQPAEAGRFKVL
TVNGYMLGSAMSRPIIHFGSDYEDRYYRENMH

FIGURE 14C

PRION CHIMERAS USING P69 CONTROL B AND HUMAN PrP (126 – 154)

P69B/Hu126-154 Chimera No. 5: His Tag - P69 Control B – HuPrP (126-154)

SEQ ID NO. 90:
MHHHHHHGGMNMSLSRIVKAAPLRRTTLAMALGALGAAPAAHADWNNQSIV
KTGERQHGIHIQGSDPGGVRTASGTTIKVSGRQAQGILLENPAAELQFRNGSVTS
SGQLSDDGIRRFLGTVTVKAGKLVADHATLANVGDTWDDDGIALYVAGEQAQA
SIADSTLQGAGGVQIERGANVTVQRSAIVDGGLHIGALQSLQPEDLPPSRVVLRD
TNVTAVPASGAPAAVSVLGASELTLDGGHITGGRAAGVAAMQGAVVHLQRATI
RRGDALAGGAVPGGAVPGGAVPGGFGPGGFGPVLDGWYGVDVSGSSVELAQSI
VEAPELGAAIRVGRGARVTVPGGSLSAPHGNVIETGGARRFAPQAAPLSITLQA
GAHAQGKALLYRVLPEPVKLTLTGGADAQGDIVATELPSIPGTSIGPLDVALASQ
ARWTGATRAVDSLSIDNATWVMTDNSNVGALRLASDGSVDFQQPAEAGRFKVL
TVGYMLGSAMSRPIIHFGSDYEDRYYRENMH

P69B/Hu126-154 Chimera No. 6: His Tag - P69 Control B – HuPrP (126-154)

SEQ ID NO. 91:
MHHHHHHGGMNMSLSRIVKAAPLRRTTLAMALGALGAAPAAHADWNNQSIV
KTGERQHGIHIQGSDPGGVRTASGTTIKVSGRQAQGILLENPAAELQFRNGSVTS
SGQLSDDGIRRFLGTVTVKAGKLVADHATLANVGDTWDDDGIALYVAGEQAQA
SIADSTLQGAGGVQIERGANVTVQRSAIVDGGLHIGALQSLQPEDLPPSRVVLRD
TNVTAVPASGAPAAVSVLGASELTLDGGHITGGRAAGVAAMQGAVVHLQRATI
RRGDALAGGAVPGGAVPGGAVPGGFGPGGFGPVLDGWYGVDVSGSSVELAQSI
VEAPELGAAIRVGRGARVTVPGGSLSAPHGNVIETGGARRFAPQAAPLSITLQA
GAHAQGKALLYRVLPEPVKLTLTGGADAQGDIVATELPSIPGTSIGPLDVALASQ
ARWTGATRAVDSLSIDNATWVMTDNSNVGALRLASDGSVDFQQPAEAGRFKVL
TGYMLGSAMSRPIIHFGSDYEDRYYRENMH

FIGURE 14D

PRION CHIMERAS USING P69 CONTROL B AND HUMAN PrP (126 – 154)

P69B/Hu126-154 Chimera No. 7: His Tag - P69 Control B – HuPrP (126-154)

SEQ ID NO. 92:
MHHHHHHGGMNMSLSRIVKAAPLRRTTLAMALGALGAAPAAHADWNNQSIV
KTGERQHGIHIQGSDPGGVRTASGTTIKVSGRQAQGILLENPAAELQFRNGSVTS
SGQLSDDGIRRFLGTVTVKAGKLVADHATLANVGDTWDDDGIALYVAGEQAQA
SIADSTLQGAGGVQIERGANVTVQRSAIVDGGLHIGALQSLQPEDLPPSRVVLRD
TNVTAVPASGAPAAVSVLGASELTLDGGHITGGRAAGVAAMQGAVVHLQRATI
RRGDALAGGAVPGGAVPGGAVPGGFGPGGFGPVLDGWYGVDVSGSSVELAQSI
VEAPELGAAIRVGRGARVTVPGGSLSAPHGNVIETGGARRFAPQAAPLSITLQA
GAHAQGKALLYRVLPEPVKLTLTGGADAQGDIVATELPSIPGTSIGPLDVALASQ
ARWTGATRAVDSLSIDNATWVMTDNSNVGALRLASDGSVDFQQPAEAGRFKVL
GYMLGSAMSRPIIHFGSDYEDRYYRENMH

P69B/Hu126-154 Chimera No. 8: His Tag - P69 Control B – HuPrP (126-154)

SEQ ID NO. 93:
MHHHHHHGGMNMSLSRIVKAAPLRRTTLAMALGALGAAPAAHADWNNQSIV
KTGERQHGIHIQGSDPGGVRTASGTTIKVSGRQAQGILLENPAAELQFRNGSVTS
SGQLSDDGIRRFLGTVTVKAGKLVADHATLANVGDTWDDDGIALYVAGEQAQA
SIADSTLQGAGGVQIERGANVTVQRSAIVDGGLHIGALQSLQPEDLPPSRVVLRD
TNVTAVPASGAPAAVSVLGASELTLDGGHITGGRAAGVAAMQGAVVHLQRATI
RRGDALAGGAVPGGAVPGGAVPGGFGPGGFGPVLDGWYGVDVSGSSVELAQSI
VEAPELGAAIRVGRGARVTVPGGSLSAPHGNVIETGGARRFAPQAAPLSITLQA
GAHAQGKALLYRVLPEPVKLTLTGGADAQGDIVATELPSIPGTSIGPLDVALASQ
ARWTGATRAVDSLSIDNATWVMTDNSNVGALRLASDGSVDFQQPAEAGRFKVG
YMLGSAMSRPIIHFGSDYEDRYYRENMH

FIGURE 15A

PRION CHIMERAS USING P69 CONTROL B AND MOUSE PrP (126-154)

P69B/Mo126-154 Chimera No. 1: His Tag – P69 Control B – Mouse PrP (126-154):

SEQ ID NO. 94:
MHHHHHHGGMNMSLSRIVKAAPLRRTTLAMALGALGAAPAAHADWNNQSIV
KTGERQHGIHIQGSDPGGVRTASGTTIKVSGRQAQGILLENPAAELQFRNGSVTS
SGQLSDDGIRRFLGTVTVKAGKLVADHATLANVGDTWDDDGIALYVAGEQAQA
SIADSTLQGAGGVQIERGANVTVQRSAIVDGGLHIGALQSLQPEDLPPSRVVLRD
TNVTAVPASGAPAAVSVLGASELTLDGGHITGGRAAGVAAMQGAVVHLQRATI
RRGDALAGGAVPGGAVPGGAVPGGFGPGGFGPVLDGWYGVDVSGSSVELAQSI
VEAPELGAAIRVGRGARVTVPGGSLSAPHGNVIETGGARRFAPQAAPLSITLQA
GAHAQGKALLYRVLPEPVKLTLTGGADAQGDIVATELPSIPGTSIGPLDVALASQ
ARWTGATRAVDSLSIDNATWVMTDNSNVGALRLASDGSVDFQQPAEAGRFKVL
TVNTLAGYMLGSAMSRPMIHFGNDWEDRYYRENMY

P69B/Mo126-154 Chimera No. 2: His Tag – P69 Control B – Mouse PrP (126-154):

SEQ ID NO. 95:
MHHHHHHGGMNMSLSRIVKAAPLRRTTLAMALGALGAAPAAHADWNNQSIV
KTGERQHGIHIQGSDPGGVRTASGTTIKVSGRQAQGILLENPAAELQFRNGSVTS
SGQLSDDGIRRFLGTVTVKAGKLVADHATLANVGDTWDDDGIALYVAGEQAQA
SIADSTLQGAGGVQIERGANVTVQRSAIVDGGLHIGALQSLQPEDLPPSRVVLRD
TNVTAVPASGAPAAVSVLGASELTLDGGHITGGRAAGVAAMQGAVVHLQRATI
RRGDALAGGAVPGGAVPGGAVPGGFGPGGFGPVLDGWYGVDVSGSSVELAQSI
VEAPELGAAIRVGRGARVTVPGGSLSAPHGNVIETGGARRFAPQAAPLSITLQA
GAHAQGKALLYRVLPEPVKLTLTGGADAQGDIVATELPSIPGTSIGPLDVALASQ
ARWTGATRAVDSLSIDNATWVMTDNSNVGALRLASDGSVDFQQPAEAGRFKVL
TVNTLGYMLGSAMSRPMIHFGNDWEDRYYRENMY

FIGURE 15B

PRION CHIMERAS USING P69 CONTROL B AND MOUSE PrP (126-154)

P69B/Mo126-154 Chimera No. 3: His Tag – P69 Control B – Mouse PrP (126-154):

SEQ ID NO. 96:
MHHHHHHGGMNMSLSRIVKAAPLRRTTLAMALGALGAAPAAHADWNNQSIV
KTGERQHGIHIQGSDPGGVRTASGTTIKVSGRQAQGILLENPAAELQFRNGSVTS
SGQLSDDGIRRFLGTVTVKAGKLVADHATLANVGDTWDDDGIALYVAGEQAQA
SIADSTLQGAGGVQIERGANVTVQRSAIVDGGLHIGALQSLQPEDLPPSRVVLRD
TNVTAVPASGAPAAVSVLGASELTLDGGHITGGRAAGVAAMQGAVVHLQRATI
RRGDALAGGAVPGGAVPGGAVPGGFGPGGFGPVLDGWYGVDVSGSSVELAQSI
VEAPELGAAIRVGRGARVTVPGGSLSAPHGNVIETGGARRFAPQAAPLSITLQA
GAHAQGKALLYRVLPEPVKLTLTGGADAQGDIVATELPSIPGTSIGPLDVALASQ
ARWTGATRAVDSLSIDNATWVMTDNSNVGALRLASDGSVDFQQPAEAGRFKVL
TVNTGYMLGSAMSRPMIHFGNDWEDRYYRENMY

P69B/Mo126-154 Chimera No. 4: His Tag – P69 Control B – Mouse PrP (126-154):

SEQ ID NO. 97:
MHHHHHHGGMNMSLSRIVKAAPLRRTTLAMALGALGAAPAAHADWNNQSIV
KTGERQHGIHIQGSDPGGVRTASGTTIKVSGRQAQGILLENPAAELQFRNGSVTS
SGQLSDDGIRRFLGTVTVKAGKLVADHATLANVGDTWDDDGIALYVAGEQAQA
SIADSTLQGAGGVQIERGANVTVQRSAIVDGGLHIGALQSLQPEDLPPSRVVLRD
TNVTAVPASGAPAAVSVLGASELTLDGGHITGGRAAGVAAMQGAVVHLQRATI
RRGDALAGGAVPGGAVPGGAVPGGFGPGGFGPVLDGWYGVDVSGSSVELAQSI
VEAPELGAAIRVGRGARVTVPGGSLSAPHGNVIETGGARRFAPQAAPLSITLQA
GAHAQGKALLYRVLPEPVKLTLTGGADAQGDIVATELPSIPGTSIGPLDVALASQ
ARWTGATRAVDSLSIDNATWVMTDNSNVGALRLASDGSVDFQQPAEAGRFKVL
TVNGYMLGSAMSRPMIHFGNDWEDRYYRENMY

FIGURE 15C

PRION CHIMERAS USING P69 CONTROL B AND MOUSE PrP (126-154)

P69B/Mo126-154 Chimera No. 5: His Tag – P69 Control B – Mouse PrP (126-154):

SEQ ID NO. 98:
MHHHHHHGGMNMSLSRIVKAAPLRRTTLAMALGALGAAPAAHADWNNQSIV
KTGERQHGIHIQGSDPGGVRTASGTTIKVSGRQAQGILLENPAAELQFRNGSVTS
SGQLSDDGIRRFLGTVTVKAGKLVADHATLANVGDTWDDDGIALYVAGEQAQA
SIADSTLQGAGGVQIERGANVTVQRSAIVDGGLHIGALQSLQPEDLPPSRVVLRD
TNVTAVPASGAPAAVSVLGASELTLDGGHITGGRAAGVAAMQGAVVHLQRATI
RRGDALAGGAVPGGAVPGGAVPGGFGPGGFGPVLDGWYGVDVSGSSVELAQSI
VEAPELGAAIRVGRGARVTVPGGSLSAPHGNVIETGGARRFAPQAAPLSITLQA
GAHAQGKALLYRVLPEPVKLTLTGGADAQGDIVATELPSIPGTSIGPLDVALASQ
ARWTGATRAVDSLSIDNATWVMTDNSNVGALRLASDGSVDFQQPAEAGRFKVL
TVGYMLGSAMSRPMIHFGNDWEDRYYRENMY

P69B/Mo126-154 Chimera No. 6: His Tag – P69 Control B – Mouse PrP (126-154):

SEQ ID NO. 99:
MHHHHHHGGMNMSLSRIVKAAPLRRTTLAMALGALGAAPAAHADWNNQSIV
KTGERQHGIHIQGSDPGGVRTASGTTIKVSGRQAQGILLENPAAELQFRNGSVTS
SGQLSDDGIRRFLGTVTVKAGKLVADHATLANVGDTWDDDGIALYVAGEQAQA
SIADSTLQGAGGVQIERGANVTVQRSAIVDGGLHIGALQSLQPEDLPPSRVVLRD
TNVTAVPASGAPAAVSVLGASELTLDGGHITGGRAAGVAAMQGAVVHLQRATI
RRGDALAGGAVPGGAVPGGAVPGGFGPGGFGPVLDGWYGVDVSGSSVELAQSI
VEAPELGAAIRVGRGARVTVPGGSLSAPHGNVIETGGARRFAPQAAPLSITLQA
GAHAQGKALLYRVLPEPVKLTLTGGADAQGDIVATELPSIPGTSIGPLDVALASQ
ARWTGATRAVDSLSIDNATWVMTDNSNVGALRLASDGSVDFQQPAEAGRFKVL
TGYMLGSAMSRPMIHFGNDWEDRYYRENMY

FIGURE 15D

PRION CHIMERAS USING P69 CONTROL B AND MOUSE PrP (126-154)

P69B/Mo126-154 Chimera No. 7: His Tag – P69 Control B – Mouse PrP (126-154):

SEQ ID NO. 100:
MHHHHHHGGMNMSLSRIVKAAPLRRTTLAMALGALGAAPAAHADWNNQSIV KTGERQHGIHIQGSDPGGVRTASGTTIKVSGRQAQGILLENPAAELQFRNGSVTS SGQLSDDGIRRFLGTVTVKAGKLVADHATLANVGDTWDDDGIALYVAGEQAQA SIADSTLQGAGGVQIERGANVTVQRSAIVDGGLHIGALQSLQPEDLPPSRVVLRD TNVTAVPASGAPAAVSVLGASELTLDGGHITGGRAAGVAAMQGAVVHLQRATI RRGDALAGGAVPGGAVPGGAVPGGFGPGGFGPVLDGWYGVDVSGSSVELAQSI VEAPELGAAIRVGRGARVTVPGGSLSAPHGNVIETGGARRFAPQAAPLSITLQA GAHAQGKALLYRVLPEPVKLTLTGGADAQGDIVATELPSIPGTSIGPLDVALASQ ARWTGATRAVDSLSIDNATWVMTDNSNVGALRLASDGSVDFQQPAEAGRFKVL GYMLGSAMSRPMIHFGNDWEDRYYRENMY

P69B/Mo126-154 Chimera No. 8: His Tag – P69 Control B – Mouse PrP (126-154):

SEQ ID NO. 101:
MHHHHHHGGMNMSLSRIVKAAPLRRTTLAMALGALGAAPAAHADWNNQSIV KTGERQHGIHIQGSDPGGVRTASGTTIKVSGRQAQGILLENPAAELQFRNGSVTS SGQLSDDGIRRFLGTVTVKAGKLVADHATLANVGDTWDDDGIALYVAGEQAQA SIADSTLQGAGGVQIERGANVTVQRSAIVDGGLHIGALQSLQPEDLPPSRVVLRD TNVTAVPASGAPAAVSVLGASELTLDGGHITGGRAAGVAAMQGAVVHLQRATI RRGDALAGGAVPGGAVPGGAVPGGFGPGGFGPVLDGWYGVDVSGSSVELAQSI VEAPELGAAIRVGRGARVTVPGGSLSAPHGNVIETGGARRFAPQAAPLSITLQA GAHAQGKALLYRVLPEPVKLTLTGGADAQGDIVATELPSIPGTSIGPLDVALASQ ARWTGATRAVDSLSIDNATWVMTDNSNVGALRLASDGSVDFQQPAEAGRFKVG YMLGSAMSRPMIHFGNDWEDRYYRENMY

FIGURE 16A

PRION CHIMERAS USING GCA CONTROL A AND HUMAN PrP (126-154)

GCAA/HuPrP126-154 Chimera No. 1: HuPrP (126-154) – GCA Control A – His Tag:

SEQ ID NO. 102:
MGYMLGSAMSRPIIHFGSDYEDRYYRENMHTAYIDPQASVIGEVTIGANVMVSP MASIRSDEGMPIFVGDRSNVQDGVVLHALETINEEGEPIEDNIVEVDGKEYAVYI GNNVSLAHQSQVHGPAAVGDDTFIGMQAFVFKSKVGNNCVLEPRSAAIGVTIPD GRYIPAGMVVTSQAEADKLPEVTDDYAYSHTNEAVVYVNVHLAEGYKETSGGH HHHHH

GCAA/HuPrP126-154 Chimera No. 2: HuPrP (126-154) – GCA Control A – His Tag:

SEQ ID NO. 103:
MGYMLGSAMSRPIIHFGSDYEDRYYRENMHAYIDPQASVIGEVTIGANVMVSPM ASIRSDEGMPIFVGDRSNVQDGVVLHALETINEEGEPIEDNIVEVDGKEYAVYIG NNVSLAHQSQVHGPAAVGDDTFIGMQAFVFKSKVGNNCVLEPRSAAIGVTIPDG RYIPAGMVVTSQAEADKLPEVTDDYAYSHTNEAVVYVNVHLAEGYKETSGGHH HHHH

GCAA/HuPrP126-154 Chimera No. 3: HuPrP (126-154) – GCA Control A – His Tag:

SEQ ID NO. 104:
MGYMLGSAMSRPIIHFGSDYEDRYYRENMHYIDPQASVIGEVTIGANVMVSPMA SIRSDEGMPIFVGDRSNVQDGVVLHALETINEEGEPIEDNIVEVDGKEYAVYIGN NVSLAHQSQVHGPAAVGDDTFIGMQAFVFKSKVGNNCVLEPRSAAIGVTIPDGR YIPAGMVVTSQAEADKLPEVTDDYAYSHTNEAVVYVNVHLAEGYKETSGGHHH HHH

FIGURE 16B

PRION CHIMERAS USING GCA CONTROL A AND HUMAN PrP (126-154)

GCAA/HuPrP126-154 Chimera No. 4: HuPrP (126-154) – GCA Control A – His Tag:

SEQ ID NO. 105:
MGYMLGSAMSRPIIHFGSDYEDRYYRENMHIDPQASVIGEVTIGANVMVSPMAS IRSDEGMPIFVGDRSNVQDGVVLHALETINEEGEPIEDNIVEVDGKEYAVYIGNN VSLAHQSQVHGPAAVGDDTFIGMQAFVFKSKVGNNCVLEPRSAAIGVTIPDGRY IPAGMVVTSQAEADKLPEVTDDYAYSHTNEAVVYVNVHLAEGYKETSGGHHHH HH

GCAA/HuPrP126-154 Chimera No. 5: HuPrP (126-154) – GCA Control A – His Tag:

SEQ ID NO. 106:
MGYMLGSAMSRPIIHFGSDYEDRYYRENMHDPQASVIGEVTIGANVMVSPMASI RSDEGMPIFVGDRSNVQDGVVLHALETINEEGEPIEDNIVEVDGKEYAVYIGNNV SLAHQSQVHGPAAVGDDTFIGMQAFVFKSKVGNNCVLEPRSAAIGVTIPDGRYIP AGMVVTSQAEADKLPEVTDDYAYSHTNEAVVYVNVHLAEGYKETSGGHHHHH H

GCAA/HuPrP126-154 Chimera No. 6: HuPrP (126-154) – GCA Control A – His Tag:

SEQ ID NO. 107:
MGYMLGSAMSRPIIHFGSDYEDRYYRENMHPQASVIGEVTIGANVMVSPMASIR SDEGMPIFVGDRSNVQDGVVLHALETINEEGEPIEDNIVEVDGKEYAVYIGNNVS LAHQSQVHGPAAVGDDTFIGMQAFVFKSKVGNNCVLEPRSAAIGVTIPDGRYIP AGMVVTSQAEADKLPEVTDDYAYSHTNEAVVYVNVHLAEGYKETSGGHHHHH H

FIGURE 17A

PRION CHIMERAS USING GCA CONTROL A AND MOUSE PrP (126 – 154)

GCAA/MoPrP126-154 Chimera No. 1: Mo PrP (126-154) – GCA Control A – His Tag:

SEQ ID NO. 108:
MGYMLGSAMSRPMIHFGNDWEDRYYRENMYTAYIDPQASVIGEVTIGANVMVS PMASIRSDEGMPIFVGDRSNVQDGVVLHALETINEEGEPIEDNIVEVDGKEYAVY IGNNVSLAHQSQVHGPAAVGDDTFIGMQAFVFKSKVGNNCVLEPRSAAIGVTIP DGRYIPAGMVVTSQAEADKLPEVTDDYAYSHTNEAVVYVNVHLAEGYKETSGG HHHHHH

GCAA/MoPrP126-154 Chimera No. 2: Mo PrP (126-154) – GCA Control A – His Tag:

SEQ ID NO. 109:
MGYMLGSAMSRPMIHFGNDWEDRYYRENMYAYIDPQASVIGEVTIGANVMVSP MASIRSDEGMPIFVGDRSNVQDGVVLHALETINEEGEPIEDNIVEVDGKEYAVYI GNNVSLAHQSQVHGPAAVGDDTFIGMQAFVFKSKVGNNCVLEPRSAAIGVTIPD GRYIPAGMVVTSQAEADKLPEVTDDYAYSHTNEAVVYVNVHLAEGYKETSGGH HHHHH

GCAA/MoPrP126-154 Chimera No. 3: Mo PrP (126-154) – GCA Control A – His Tag:

SEQ ID NO. 110:
MGYMLGSAMSRPMIHFGNDWEDRYYRENMYYIDPQASVIGEVTIGANVMVSP MASIRSDEGMPIFVGDRSNVQDGVVLHALETINEEGEPIEDNIVEVDGKEYAVYI GNNVSLAHQSQVHGPAAVGDDTFIGMQAFVFKSKVGNNCVLEPRSAAIGVTIPD GRYIPAGMVVTSQAEADKLPEVTDDYAYSHTNEAVVYVNVHLAEGYKETSGGH HHHHH

FIGURE 17B

PRION CHIMERAS USING GCA CONTROL A AND MOUSE PrP (126 – 154)

GCAA/MoPrP126-154 Chimera No. 4: Mo PrP (126-154) – GCA Control A – His Tag:

SEQ ID NO. 111:
MGYMLGSAMSRPMIHFGNDWEDRYYRENMYIDPQASVIGEVTIGANVMVSPM ASIRSDEGMPIFVGDRSNVQDGVVLHALETINEEGEPIEDNIVEVDGKEYAVYIG NNVSLAHQSQVHGPAAVGDDTFIGMQAFVFKSKVGNNCVLEPRSAAIGVTIPDG RYIPAGMVVTSQAEADKLPEVTDDYAYSHTNEAVVYVNVHLAEGYKETSGGHH HHHH

GCAA/MoPrP126-154 Chimera No. 5: Mo PrP (126-154) – GCA Control A – His Tag:

SEQ ID NO. 112:
MGYMLGSAMSRPMIHFGNDWEDRYYRENMYDPQASVIGEVTIGANVMVSPMA SIRSDEGMPIFVGDRSNVQDGVVLHALETINEEGEPIEDNIVEVDGKEYAVYIGN NVSLAHQSQVHGPAAVGDDTFIGMQAFVFKSKVGNNCVLEPRSAAIGVTIPDGR YIPAGMVVTSQAEADKLPEVTDDYAYSHTNEAVVYVNVHLAEGYKETSGGHHH HHH

GCAA/MoPrP126-154 Chimera No. 6: Mo PrP (126-154) – GCA Control A – His Tag:

SEQ ID NO. 113:
MGYMLGSAMSRPMIHFGNDWEDRYYRENMYPQASVIGEVTIGANVMVSPMASI RSDEGMPIFVGDRSNVQDGVVLHALETINEEGEPIEDNIVEVDGKEYAVYIGNNV SLAHQSQVHGPAAVGDDTFIGMQAFVFKSKVGNNCVLEPRSAAIGVTIPDGRYIP AGMVVTSQAEADKLPEVTDDYAYSHTNEAVVYVNVHLAEGYKETSGGHHHHH H

FIGURE 18A

PRION CHIMERAS USING GCA CONTROL B AND HUMAN PrP (126-154)

GCAB/HuPrP126-154 Chimera No. 1: His Tag – GCA Control B – Hu PrP (126-154):

SEQ ID NO. 114:

MHHHHHHGGMMFNKQIFTILILSLSLALAGSGCISEGAEDNVAQEITVDEFSNI
RENPVTPWNPEPSAPVIDPTAYIDPQASVIGEVTIGANVMVSPMASIRSDEGMPIFVGD
RSNVQDGVVLHALETINEEGEPIEDNIVEVDGKEYAVYIGNNVSLAHQSQVHGPAAV
GDDTFIGMQAFVFKSKVGNNCVLEPRSAAIGVTIPDGRYIPAGMVVT**GYMLGSAMS**
RPIIHFGSDYEDRYYRENMH

GCAB/HuPrP126-154 Chimera No. 2: His Tag – GCA Control B – Hu PrP (126-154):

SEQ ID NO. 115:

MHHHHHHGGMMFNKQIFTILILSLSLALAGSGCISEGAEDNVAQEITVDEFSNI
RENPVTPWNPEPSAPVIDPTAYIDPQASVIGEVTIGANVMVSPMASIRSDEGMPIFVGD
RSNVQDGVVLHALETINEEGEPIEDNIVEVDGKEYAVYIGNNVSLAHQSQVHGPAAV
GDDTFIGMQAFVFKSKVGNNCVLEPRSAAIGVTIPDGRYIPAGMVV**GYMLGSAMSR**
PIIHFGSDYEDRYYRENMH

GCAB/HuPrP126-154 Chimera No. 3: His Tag – GCA Control B – Hu PrP (126-154):

SEQ ID NO. 116:

MHHHHHHGGMMFNKQIFTILILSLSLALAGSGCISEGAEDNVAQEITVDEFSNI
RENPVTPWNPEPSAPVIDPTAYIDPQASVIGEVTIGANVMVSPMASIRSDEGMPIFVGD
RSNVQDGVVLHALETINEEGEPIEDNIVEVDGKEYAVYIGNNVSLAHQSQVHGPAAV
GDDTFIGMQAFVFKSKVGNNCVLEPRSAAIGVTIPDGRYIPAGMV**GYMLGSAMSRP**
IIHFGSDYEDRYYRENMH

FIGURE 18B

PRION CHIMERAS USING GCA CONTROL B AND HUMAN PrP (126-154)

GCAB/HuPrP126-154 Chimera No. 4: His Tag – GCA Control B – Hu PrP (126-154):

SEQ ID NO. 117:

MHHHHHHGGMMFNKQIFTILILSLSLALAGSGCISEGAEDNVAQEITVDEFSNI
RENPVTPWNPEPSAPVIDPTAYIDPQASVIGEVTIGANVMVSPMASIRSDEGMPIFVGD
RSNVQDGVVLHALETINEEGEPIEDNIVEVDGKEYAVYIGNNVSLAHQSQVHGPAAV
GDDTFIGMQAFVFKSKVGNNCVLEPRSAAIGVTIPDGRYIPAGM**GYMLGSAMSRPII**
HFGSDYEDRYYRENMH

GCAB/HuPrP126-154 Chimera No. 5: His Tag – GCA Control B – Hu PrP (126-154):

SEQ ID NO. 118:

MHHHHHHGGMMFNKQIFTILILSLSLALAGSGCISEGAEDNVAQEITVDEFSNI
RENPVTPWNPEPSAPVIDPTAYIDPQASVIGEVTIGANVMVSPMASIRSDEGMPIFVGD
RSNVQDGVVLHALETINEEGEPIEDNIVEVDGKEYAVYIGNNVSLAHQSQVHGPAAV
GDDTFIGMQAFVFKSKVGNNCVLEPRSAAIGVTIPDGRYIPAG**GYMLGSAMSRPIIH**
FGSDYEDRYYRENMH

GCAB/HuPrP126-154 Chimera No. 6: His Tag – GCA Control B – Hu PrP (126-154):

SEQ ID NO. 119:

MHHHHHHGGMMFNKQIFTILILSLSLALAGSGCISEGAEDNVAQEITVDEFSNI
RENPVTPWNPEPSAPVIDPTAYIDPQASVIGEVTIGANVMVSPMASIRSDEGMPIFVGD
RSNVQDGVVLHALETINEEGEPIEDNIVEVDGKEYAVYIGNNVSLAHQSQVHGPAAV
GDDTFIGMQAFVFKSKVGNNCVLEPRSAAIGVTIPDGRYIPA**GYMLGSAMSRPIIHF**
GSDYEDRYYRENMH

FIGURE 19A

PRION CHIMERAS USING GCA CONTROL B AND MOUSE PrP (126 – 154)

GCAB/MoPrP126-154 Chimera No. 1: His Tag – GCA Control B – Mo PrP (126 – 154):

SEQ ID NO. 120:

MHHHHHHGGMMFNKQIFTILILSLSLALAGSGCISEGAEDNVAQEITVDEFSNI
RENPVTPWNPEPSAPVIDPTAYIDPQASVIGEVTIGANVMVSPMASIRSDEGMPIFVGD
RSNVQDGVVLHALETINEEGEPIEDNIVEVDGKEYAVYIGNNVSLAHQSQVHGPAAV
GDDTFIGMQAFVFKSKVGNNCVLEPRSAAIGVTIPDGRYIPAGMVVTMGYMLGSA
MSRPMIHFGNDWEDRYYRENMY

GCAB/MoPrP126 – 154 Chimera No. 2: His Tag – GCA Control B – Mo PrP (126 – 154):

SEQ ID NO. 121:

MHHHHHHGGMMFNKQIFTILILSLSLALAGSGCISEGAEDNVAQEITVDEFSNI
RENPVTPWNPEPSAPVIDPTAYIDPQASVIGEVTIGANVMVSPMASIRSDEGMPIFVGD
RSNVQDGVVLHALETINEEGEPIEDNIVEVDGKEYAVYIGNNVSLAHQSQVHGPAAV
GDDTFIGMQAFVFKSKVGNNCVLEPRSAAIGVTIPDGRYIPAGMVVT**GYMLGSAMS**
RPMIHFGNDWEDRYYRENMY

GCAB/MoPrP126 – 154 Chimera No. 3: His Tag – GCA Control B – Mo PrP (126 – 154):

SEQ ID NO. 122:

MHHHHHHGGMMFNKQIFTILILSLSLALAGSGCISEGAEDNVAQEITVDEFSNI
RENPVTPWNPEPSAPVIDPTAYIDPQASVIGEVTIGANVMVSPMASIRSDEGMPIFVGD
RSNVQDGVVLHALETINEEGEPIEDNIVEVDGKEYAVYIGNNVSLAHQSQVHGPAAV
GDDTFIGMQAFVFKSKVGNNCVLEPRSAAIGVTIPDGRYIPAGMVV**GYMLGSAMSR**
PMIHFGNDWEDRYYRENMY

FIGURE 19B

PRION CHIMERAS USING GCA CONTROL B AND MOUSE PrP (126 – 154)

GCAB/MoPrP126 – 154 Chimera No. 4: His Tag – GCA Control B – Mo PrP (126 – 154):

SEQ ID NO. 123:

MHHHHHHGGMMFNKQIFTILILSLSLALAGSGCISEGAEDNVAQEITVDEFSNI
RENPVTPWNPEPSAPVIDPTAYIDPQASVIGEVTIGANVMVSPMASIRSDEGMPIFVGD
RSNVQDGVVLHALETINEEGEPIEDNIVEVDGKEYAVYIGNNVSLAHQSQVHGPAAV
GDDTFIGMQAFVFKSKVGNNCVLEPRSAAIGVTIPDGRYIPAGMV**GYMLGSAMSRP**
MIHFGNDWEDRYYRENMY

GCAB/MoPrP126 – 154 Chimera No. 5: His Tag – GCA Control B – Mo PrP (126 – 154):

SEQ ID NO. 124:

MHHHHHHGGMMFNKQIFTILILSLSLALAGSGCISEGAEDNVAQEITVDEFSNI
RENPVTPWNPEPSAPVIDPTAYIDPQASVIGEVTIGANVMVSPMASIRSDEGMPIFVGD
RSNVQDGVVLHALETINEEGEPIEDNIVEVDGKEYAVYIGNNVSLAHQSQVHGPAAV
GDDTFIGMQAFVFKSKVGNNCVLEPRSAAIGVTIPDGRYIPAGM**GYMLGSAMSRPM**
IHFGNDWEDRYYRENMY

GCAB/MoPrP126 – 154 Chimera No. 6: His Tag – GCA Control B – Mo PrP (126 – 154):

SEQ ID NO. 125:

MHHHHHHGGMMFNKQIFTILILSLSLALAGSGCISEGAEDNVAQEITVDEFSNI
RENPVTPWNPEPSAPVIDPTAYIDPQASVIGEVTIGANVMVSPMASIRSDEGMPIFVGD
RSNVQDGVVLHALETINEEGEPIEDNIVEVDGKEYAVYIGNNVSLAHQSQVHGPAAV
GDDTFIGMQAFVFKSKVGNNCVLEPRSAAIGVTIPDGRYIPAG**GYMLGSAMSRPMI**
HFGNDWEDRYYRENMY

FIGURE 20A
DNA CONSTRUCTS ENCODING PRION CHIMERAS

Construct designated Tpa.p69.mprpf1 (SEQ ID NO:155):

GAATTCGCCACCATGGATGCAATGAAGAGAGGGCTCTGCTGTGTGCTGCTGCTGT
GTGGAGCAGTCTTCGTTTCGCCCAGCGCTAGCATGAACATGTCTCTGTCACGCAT
TGTCAAGGCGGCGCCCCTGCGCCGCACCACGCTGGCCATGGCGCTGGGCGCGCT
GGGCGCCGCCCCGGCGGCGCATGCCGACTGGAACAACCAGTCCATCGTCAAGAC
CGGTGAGCGCCAGCATGGCATCCATATCCAGGGCTCCGACCCGGGCGGCGTACG
GACCGCCAGCGGAACCACCATCAAGGTAAGCGGCCGTCAGGCCCAGGGCATCCT
GCTAGAAAATCCCGCGGCCGAGCTGCAGTTCCGGAACGGCAGTGTCACGTCGTC
GGGACAGTTGTCCGACGATGGCATCCGGCGCTTTCTGGGCACCGTCACCGTCAAG
GCCGGCAAGCTGGTCGCCGATCACGCCACGCTGGCCAACGTTGGCGACACCTGG
GACGACGACGGCATCGCGCTCTATGTGGCCGGCGAACAGGCCCAGGCCAGCATC
GCCGACAGCACCCTGCAGGGCGCTGGCGGCGTGCAGATCGAGCGCGGCGCCAAT
GTCACGGTCCAACGCAGCGCCATCGTCGACGGGGGCTTGCATATCGGCGCCCTGC
AGTCATTGCAGCCGGAAGACCTTCCGCCCAGCCGGGTGGTGCTGCGCGACACCA
ACGTGACCGCCGTGCCCGCCAGCGGCGCGCCCGCGGCGGTGTCTGTGTTGGGGG
CCAGTGAGCTTACGCTCGACGGCGGGCACATCACCGGCGGGCGGGCAGCGGGGG
TGGCGGCCATGCAAGGGGCGGTCGTGCATCTGCAGCGCGCGACGATACGGCGCG
GGGACGCGCCTGCCGGCGGTGCGGTTCCCGGCGGTGCGGTTCCCGGTGGTGCGG
TTCCCGGCGGCTTCGGTCCCGGCGGCTTCGGTCCCGTCCTCGACGGCTGGTATGG
CGTGGACGTATCGGGCTCCAGCGTGGAGCTCGCCCAGTCGATCGTCGAGGCGCC
GGAGCTGGGCGCCGCAATCCGGGTGGGCCGCGGCGCCAGGGTGACGGTGTCGGG
CGGCAGCTTGTCCGCACCGCACGGCAATGTCATCGAGACCGGCGGCGCGCGTCG
CTTTGCGCCTCAAGCCGCGCCCCTGTCGATCACCTTGCAGGCCGGCGCGCATGCC
CAGGGGAAAGCGCTGCTGTACCGGGTCCTGCCGGAGCCCGTGAAGCTGACGCTG
ACCGGGGGCGCCGATGCGCAGGGCGACATCGTCGCGACGGAGCTGCCCTCCATT
CCCGGCACGTCGATCGGGCCGCTCGACGTGGCGCTGGCCAGCCAGGCCCGATGG
ACGGGCGCTACCCGCGCGGTCGACTCGCTGTCCATCGACAACGCCACCTGGGTCA
TGACGGACAACTCGAACGTCGGTGCGCTACGGCTGGCCAGCGACGGCAGCGTCG
ATTTCCAGCAGCCGGCCGAAGCTGGGCGGTTCAAGGTCCTGACGGTCAATACGCT
GGCGAGGCCCATGATACATTTCGGCAATGACTGGGAGGACCGTTACTATCGTGA
AAACATGTATCGTTAGGATCC

Prion chimera encoded by construct designated Tpa.p69.mprpf1 (SEQ ID NO:126):

MDAMKRGLCCVLLLCGAVFVSPSASMNMSLSRIVKAAPLRRTTLAMALGALGAAP
AAHADWNNQSIVKTGERQHGIHIQGSDPGGVRTASGTTIKVSGRQAQGILLENPAAE
LQFRNGSVTSSGQLSDDGIRRFLGTVTVKAGKLVADHATLANVGDTWDDDGIALYV
AGEQAQASIADSTLQGAGGVQIERGANVTVQRSAIVDGGLHIGALQSLQPEDLPPSR
VVLRDTNVTAVPASGAPAAVSVLGASELTLDGGHITGGRAAGVAAMQGAVVHLQR
ATIRRGDAPAGGAVPGGAVPGGAVPGGFGPGGFGPVLDGWYGVDVSGSSVELAQSI
VEAPELGAAIRVGRGARVTVSGGSLSAPHGNVIETGGARRFAPQAAPLSITLQAGAH
AQGKALLYRVLPEPVKLTLTGGADAQGDIVATELPSIPGTSIGPLDVALASQARWTG
ATRAVDSLSIDNATWVMTDNSNVGALRLASDGSVDFQQPAEAGRFKVLTVNTLARP
MIHFGNDWEDRYYRENMYRO

FIGURE 20B
DNA CONSTRUCTS ENCODING PRION CHIMERAS

Construct designated Tpa.p69.mprpf2 (SEQ ID NO:156):

GAATTCGCCACCATGGATGCAATGAAGAGAGGGCTCTGCTGTGTGCTGCTGCTGT
GTGGAGCAGTCTTCGTTTCGCCCAGCGCTAGCATGAACATGTCTCTGTCACGCAT
TGTCAAGGCGGCGCCCCTGCGCCGCACCACGCTGGCCATGGCGCTGGGCGCGCT
GGGCGCCGCCCCGGCGGCGCATGCCGACTGGAACAACCAGTCCATCGTCAAGAC
CGGTGAGCGCCAGCATGGCATCCATATCCAGGGCTCCGACCCGGGCGGCGTACG
GACCGCCAGCGGAACCACCATCAAGGTAAGCGGCCGTCAGGCCCAGGGCATCCT
GCTAGAAAATCCCGCGGCCGAGCTGCAGTTCCGGAACGGCAGTGTCACGTCGTC
GGGACAGTTGTCCGACGATGGCATCCGGCGCTTTCTGGGCACCGTCACCGTCAAG
GCCGGCAAGCTGGTCGCCGATCACGCCACGCTGGCCAACGTTGGCGACACCTGG
GACGACGACGGCATCGCGCTCTATGTGGCCGGCGAACAGGCCCAGGCCAGCATC
GCCGACAGCACCCTGCAGGGCGCTGGCGGCGTGCAGATCGAGCGCGGCGCCAAT
GTCACGGTCCAACGCAGCGCCATCGTCGACGGGGGCTTGCATATCGGCGCCCTGC
AGTCATTGCAGCCGGAAGACCTTCCGCCCAGCCGGGTGGTGCTGCGCGACACCA
ACGTGACCGCCGTGCCCGCCAGCGGCGCGCCCGCGGCGGTGTCTGTGTTGGGGG
CCAGTGAGCTTACGCTCGACGGCGGGCACATCACCGGCGGGCGGGCAGCGGGGG
TGGCGGCCATGCAAGGGGCGGTCGTGCATCTGCAGCGCGCGACGATACGGCGCG
GGGACGCGCCTGCCGGCGGTGCGGTTCCCGGCGGTGCGGTTCCCGGTGGTGCGG
TTCCCGGCGGCTTCGGTCCCGGCGGCTTCGGTCCCGTCCTCGACGGCTGGTATGG
CGTGGACGTATCGGGCTCCAGCGTGGAGCTCGCCCAGTCGATCGTCGAGGCGCC
GGAGCTGGGCGCCGCAATCCGGGTGGGCCGCGGCGCCAGGGTGACGGTGTCGGG
CGGCAGCTTGTCCGCACCGCACGGCAATGTCATCGAGACCGGCGGCGCGCGTCG
CTTTGCGCCTCAAGCCGCGCCCCTGTCGATCACCTTGCAGGCCGGCGCGCATGCC
CAGGGGAAAGCGCTGCTGTACCGGGTCCTGCCGGAGCCCGTGAAGCTGACGCTG
ACCGGGGGCGCCGATGCGCAGGGCGACATCGTCGCGACGGAGCTGCCCTCCATT
CCCGGCACGTCGATCGGGCCGCTCGACGTGGCGCTGGCCAGCCAGGCCCGATGG
ACGGGCGCTACCCGCGCGGTCGACTCGCTGTCCATCGACAACGCCACCTGGGTCA
TGACGGACAACTCGAACGTCGGTGCGCTACGGCTGGCCAGCGACGGCAGCGTCG
ATTTCCAGCAGCCGGCCGAAGCTGGGCGGTTCAAGGTCCTGACGGTCAATACGCT
GAGGCCCATGATACATTTCGGCAATGACTGGGAGGACCGTTACTATCGTGAAAA
CATGTATCGTTAGGATCC

Prion chimera encoded by construct designated Tpa.p69.mprpf2 (SEQ ID NO:127):

MDAMKRGLCCVLLLCGAVFVSPSASMNMSLSRIVKAAPLRRTTLAMALGALGAAP
AAHADWNNQSIVKTGERQHGIHIQGSDPGGVRTASGTTIKVSGRQAQGILLENPAAE
LQFRNGSVTSSGQLSDDGIRRFLGTVTVKAGKLVADHATLANVGDTWDDDGIALYV
AGEQAQASIADSTLQGAGGVQIERGANVTVQRSAIVDGGLHIGALQSLQPEDLPPSR
VVLRDTNVTAVPASGAPAAVSVLGASELTLDGGHITGGRAAGVAAMQGAVVHLQR
ATIRRGDAPAGGAVPGGAVPGGAVPGGFGPGGFGPVLDGWYGVDVSGSSVELAQSI
VEAPELGAAIRVGRGARVTVSGGSLSAPHGNVIETGGARRFAPQAAPLSITLQAGAH
AQGKALLYRVLPEPVKLTLTGGADAQGDIVATELPSIPGTSIGPLDVALASQARWTG
ATRAVDSLSIDNATWVMTDNSNVGALRLASDGSVDFQQPAEAGRFKVLTVNTLRP
MIHFGNDWEDRYYRENMYRO

FIGURE 20C
DNA CONSTRUCTS ENCODING PRION CHIMERAS

Construct designated Tpa.p69.mprpf3 (SEQ ID NO:157):

GAATTCGCCACCATGGATGCAATGAAGAGAGGGCTCTGCTGTGTGCTGCTGCTGT
GTGGAGCAGTCTTCGTTTCGCCCAGCGCTAGCATGAACATGTCTCTGTCACGCAT
TGTCAAGGCGGCGCCCCTGCGCCGCACCACGCTGGCCATGGCGCTGGGCGCGCT
GGGCGCCGCCCCGGCGGCGCATGCCGACTGGAACAACCAGTCCATCGTCAAGAC
CGGTGAGCGCCAGCATGGCATCCATATCCAGGGCTCCGACCCGGGCGGCGTACG
GACCGCCAGCGGAACCACCATCAAGGTAAGCGGCCGTCAGGCCCAGGGCATCCT
GCTAGAAAATCCCGCGGCCGAGCTGCAGTTCCGGAACGGCAGTGTCACGTCGTC
GGGACAGTTGTCCGACGATGGCATCCGGCGCTTTCTGGGCACCGTCACCGTCAAG
GCCGGCAAGCTGGTCGCCGATCACGCCACGCTGGCCAACGTTGGCGACACCTGG
GACGACGACGGCATCGCGCTCTATGTGGCCGGCGAACAGGCCCAGGCCAGCATC
GCCGACAGCACCCTGCAGGGCGCTGGCGGCGTGCAGATCGAGCGCGGCGCCAAT
GTCACGGTCCAACGCAGCGCCATCGTCGACGGGGGCTTGCATATCGGCGCCCTGC
AGTCATTGCAGCCGGAAGACCTTCCGCCCAGCCGGGTGGTGCTGCGCGACACCA
ACGTGACCGCCGTGCCCGCCAGCGGCGCGCCCGCGGCGGTGTCTGTGTTGGGGG
CCAGTGAGCTTACGCTCGACGGCGGGCACATCACCGGCGGGCGGGCAGCGGGGG
TGGCGGCCATGCAAGGGGCGGTCGTGCATCTGCAGCGCGCGACGATACGGCGCG
GGGACGCGCCTGCCGGCGGTGCGGTTCCCGGCGGTGCGGTTCCCGGTGGTGCGG
TTCCCGGCGGCTTCGGTCCCGGCGGCTTCGGTCCCGTCCTCGACGGCTGGTATGG
CGTGGACGTATCGGGCTCCAGCGTGGAGCTCGCCCAGTCGATCGTCGAGGCGCC
GGAGCTGGGCGCCGCAATCCGGGTGGGCCGCGGCGCCAGGGTGACGGTGTCGGG
CGGCAGCTTGTCCGCACCGCACGGCAATGTCATCGAGACCGGCGGCGCGCGTCG
CTTTGCGCCTCAAGCCGCGCCCCTGTCGATCACCTTGCAGGCCGGCGCGCATGCC
CAGGGGAAAGCGCTGCTGTACCGGGTCCTGCCGGAGCCCGTGAAGCTGACGCTG
ACCGGGGGCGCCGATGCGCAGGGCGACATCGTCGCGACGGAGCTGCCCTCCATT
CCCGGCACGTCGATCGGGCCGCTCGACGTGGCGCTGGCCAGCCAGGCCCGATGG
ACGGGCGCTACCCGCGCGGTCGACTCGCTGTCCATCGACAACGCCACCTGGGTCA
TGACGGACAACTCGAACGTCGGTGCGCTACGGCTGGCCAGCGACGGCAGCGTCG
ATTTCCAGCAGCCGGCCGAAGCTGGGCGGTTCAAGGTCCTGACGGTCAATACGA
GGCCCATGATACATTTCGGCAATGACTGGGAGGACCGTTACTATCGTGAAAACAT
GTATCGTTAGGATCC

Prion chimera encoded by construct designated Tpa.p69.mprpf3 (SEQ ID NO:128):

MDAMKRGLCCVLLLCGAVFVSPSASMNMSLSRIVKAAPLRRTTLAMALGALGAAP
AAHADWNNQSIVKTGERQHGIHIQGSDPGGVRTASGTTIKVSGRQAQGILLENPAAE
LQFRNGSVTSSGQLSDDGIRRFLGTVTVKAGKLVADHATLANVGDTWDDDGIALYV
AGEQAQASIADSTLQGAGGVQIERGANVTVQRSAIVDGGLHIGALQSLQPEDLPPSR
VVLRDTNVTAVPASGAPAAVSVLGASELTLDGGHITGGRAAGVAAMQGAVVHLQR
ATIRRGDAPAGGAVPGGAVPGGAVPGGFGPGGFGPVLDGWYGVDVSGSSVELAQSI
VEAPELGAAIRVGRGARVTVSGGSLSAPHGNVIETGGARRFAPQAAPLSITLQAGAH
AQGKALLYRVLPEPVKLTLTGGADAQGDIVATELPSIPGTSIGPLDVALASQARWTG
ATRAVDSLSIDNATWVMTDNSNVGALRLASDGSVDFQQPAEAGRFKVLTVNTRPMI
HFGNDWEDRYYRENMYRO

FIGURE 20D
DNA CONSTRUCTS ENCODING PRION CHIMERAS

Construct designated Tpa.p69.mprpf4 (SEQ ID NO:158):

GAATTCGCCACCATGGATGCAATGAAGAGAGGGCTCTGCTGTGTGCTGCTGCTGT
GTGGAGCAGTCTTCGTTTCGCCCAGCGCTAGCATGAACATGTCTCTGTCACGCAT
TGTCAAGGCGGCGCCCCTGCGCCGCACCACGCTGGCCATGGCGCTGGGCGCGCT
GGGCGCCGCCCCGGCGGCGCATGCCGACTGGAACAACCAGTCCATCGTCAAGAC
CGGTGAGCGCCAGCATGGCATCCATATCCAGGGCTCCGACCCGGGCGGCGTACG
GACCGCCAGCGGAACCACCATCAAGGTAAGCGGCCGTCAGGCCCAGGGCATCCT
GCTAGAAAATCCCGCGGCCGAGCTGCAGTTCCGGAACGGCAGTGTCACGTCGTC
GGGACAGTTGTCCGACGATGGCATCCGGCGCTTTCTGGGCACCGTCACCGTCAAG
GCCGGCAAGCTGGTCGCCGATCACGCCACGCTGGCCAACGTTGGCGACACCTGG
GACGACGACGGCATCGCGCTCTATGTGGCCGGCGAACAGGCCCAGGCCAGCATC
GCCGACAGCACCCTGCAGGGCGCTGGCGGCGTGCAGATCGAGCGCGGCGCCAAT
GTCACGGTCCAACGCAGCGCCATCGTCGACGGGGGCTTGCATATCGGCGCCCTGC
AGTCATTGCAGCCGGAAGACCTTCCGCCCAGCCGGGTGGTGCTGCGCGACACCA
ACGTGACCGCCGTGCCCGCCAGCGGCGCGCCCGCGGCGGTGTCTGTGTTGGGGG
CCAGTGAGCTTACGCTCGACGGCGGGCACATCACCGGCGGGCGGGCAGCGGGGG
TGGCGGCCATGCAAGGGGCGGTCGTGCATCTGCAGCGCGCGACGATACGGCGCG
GGGACGCGCCTGCCGGCGGTGCGGTTCCCGGCGGTGCGGTTCCCGGTGGTGCGG
TTCCCGGCGGCTTCGGTCCCGGCGGCTTCGGTCCCGTCCTCGACGGCTGGTATGG
CGTGGACGTATCGGGCTCCAGCGTGGAGCTCGCCCAGTCGATCGTCGAGGCGCC
GGAGCTGGGCGCCGCAATCCGGGTGGGCCGCGGCGCCAGGGTGACGGTGTCGGG
CGGCAGCTTGTCCGCACCGCACGGCAATGTCATCGAGACCGGCGGCGCGCGTCG
CTTTGCGCCTCAAGCCGCGCCCCTGTCGATCACCTTGCAGGCCGGCGCGCATGCC
CAGGGGAAAGCGCTGCTGTACCGGGTCCTGCCGGAGCCCGTGAAGCTGACGCTG
ACCGGGGGCGCCGATGCGCAGGGCGACATCGTCGCGACGGAGCTGCCCTCCATT
CCCGGCACGTCGATCGGGCCGCTCGACGTGGCGCTGGCCAGCCAGGCCCGATGG
ACGGGCGCTACCCGCGCGGTCGACTCGCTGTCCATCGACAACGCCACCTGGGTCA
TGACGGACAACTCGAACGTCGGTGCGCTACGGCTGGCCAGCGACGGCAGCGTCG
ATTTCCAGCAGCCGGCCGAAGCTGGGCGGTTCAAGGTCCTGACGGTCAATAGGC
CCATGATACATTTCGGCAATGACTGGGAGGACCGTTACTATCGTGAAAACATGTA
TCGTTAGGATCC

Prion chimera encoded by construct designated Tpa.p69.mprpf4 (SEQ ID NO:129):

MDAMKRGLCCVLLLCGAVFVSPSASMNMSLSRIVKAAPLRRTTLAMALGALGAAP
AAHADWNNQSIVKTGERQHGIHIQGSDPGGVRTASGTTIKVSGRQAQGILLENPAAE
LQFRNGSVTSSGQLSDDGIRRFLGTVTVKAGKLVADHATLANVGDTWDDDGIALYV
AGEQAQASIADSTLQGAGGVQIERGANVTVQRSAIVDGGLHIGALQSLQPEDLPPSR
VVLRDTNVTAVPASGAPAAVSVLGASELTLDGGHITGGRAAGVAAMQGAVVHLQR
ATIRRGDAPAGGAVPGGAVPGGAVPGGFGPGGFGPVLDGWYGVDVSGSSVELAQSI
VEAPELGAAIRVGRGARVTVSGGSLSAPHGNVIETGGARRFAPQAAPLSITLQAGAH
AQGKALLYRVLPEPVKLTLTGGADAQGDIVATELPSIPGTSIGPLDVALASQARWTG
ATRAVDSLSIDNATWVMTDNSNVGALRLASDGSVDFQQPAEAGRFKVLTVNRPMIH
FGNDWEDRYYRENMYRO

FIGURE 20E
DNA CONSTRUCTS ENCODING PRION CHIMERAS

Construct designated Tpa.p69.mprpf5 (SEQ ID NO:159):

GAATTCGCCACCATGGATGCAATGAAGAGAGGGCTCTGCTGTGTGCTGCTGCTGT
GTGGAGCAGTCTTCGTTTCGCCCAGCGCTAGCATGAACATGTCTCTGTCACGCAT
TGTCAAGGCGGCGCCCCTGCGCCGCACCACGCTGGCCATGGCGCTGGGCGCGCT
GGGCGCCGCCCCGGCGGCGCATGCCGACTGGAACAACCAGTCCATCGTCAAGAC
CGGTGAGCGCCAGCATGGCATCCATATCCAGGGCTCCGACCCGGGCGGCGTACG
GACCGCCAGCGGAACCACCATCAAGGTAAGCGGCCGTCAGGCCCAGGGCATCCT
GCTAGAAAATCCCGCGGCCGAGCTGCAGTTCCGGAACGGCAGTGTCACGTCGTC
GGGACAGTTGTCCGACGATGGCATCCGGCGCTTTCTGGGCACCGTCACCGTCAAG
GCCGGCAAGCTGGTCGCCGATCACGCCACGCTGGCCAACGTTGGCGACACCTGG
GACGACGACGGCATCGCGCTCTATGTGGCCGGCGAACAGGCCCAGGCCAGCATC
GCCGACAGCACCCTGCAGGGCGCTGGCGGCGTGCAGATCGAGCGCGGCGCCAAT
GTCACGGTCCAACGCAGCGCCATCGTCGACGGGGGCTTGCATATCGGCGCCCTGC
AGTCATTGCAGCCGGAAGACCTTCCGCCCAGCCGGGTGGTGCTGCGCGACACCA
ACGTGACCGCCGTGCCCGCCAGCGGCGCGCCCGCGGCGGTGTCTGTGTTGGGGG
CCAGTGAGCTTACGCTCGACGGCGGGCACATCACCGGCGGGCGGGCAGCGGGGG
TGGCGGCCATGCAAGGGGCGGTCGTGCATCTGCAGCGCGCGACGATACGGCGCG
GGGACGCGCCTGCCGGCGGTGCGGTTCCCGGCGGTGCGGTTCCCGGTGGTGCGG
TTCCCGGCGGCTTCGGTCCCGGCGGCTTCGGTCCCGTCCTCGACGGCTGGTATGG
CGTGGACGTATCGGGCTCCAGCGTGGAGCTCGCCCAGTCGATCGTCGAGGCGCC
GGAGCTGGGCGCCGCAATCCGGGTGGGCCGCGGCGCCAGGGTGACGGTGTCGGG
CGGCAGCTTGTCCGCACCGCACGGCAATGTCATCGAGACCGGCGGCGCGCGTCG
CTTTGCGCCTCAAGCCGCGCCCCTGTCGATCACCTTGCAGGCCGGCGCGCATGCC
CAGGGGAAAGCGCTGCTGTACCGGGTCCTGCCGGAGCCCGTGAAGCTGACGCTG
ACCGGGGGCGCCGATGCGCAGGGCGACATCGTCGCGACGGAGCTGCCCTCCATT
CCCGGCACGTCGATCGGGCCGCTCGACGTGGCGCTGGCCAGCCAGGCCCGATGG
ACGGGCGCTACCCGCGCGGTCGACTCGCTGTCCATCGACAACGCCACCTGGGTCA
TGACGGACAACTCGAACGTCGGTGCGCTACGGCTGGCCAGCGACGGCAGCGTCG
ATTTCCAGCAGCCGGCCGAAGCTGGGCGGTTCAAGGTCCTGACGGTCAGGCCCA
TGATACATTTCGGCAATGACTGGGAGGACCGTTACTATCGTGAAAACATGTATCG
TTAGGATCC

Prion chimera encoded by construct designated Tpa.p69.mprpf5 (SEQ ID NO:130):

MDAMKRGLCCVLLLCGAVFVSPSASMNMSLSRIVKAAPLRRTTLAMALGALGAAP
AAHADWNNQSIVKTGERQHGIHIQGSDPGGVRTASGTTIKVSGRQAQGILLENPAAE
LQFRNGSVTSSGQLSDDGIRRFLGTVTVKAGKLVADHATLANVGDTWDDDGIALYV
AGEQAQASIADSTLQGAGGVQIERGANVTVQRSAIVDGGLHIGALQSLQPEDLPPSR
VVLRDTNVTAVPASGAPAAVSVLGASELTLDGGHITGGRAAGVAAMQGAVVHLQR
ATIRRGDAPAGGAVPGGAVPGGAVPGGFGPGGFGPVLDGWYGVDVSGSSVELAQSI
VEAPELGAAIRVGRGARVTVSGGSLSAPHGNVIETGGARRFAPQAAPLSITLQAGAH
AQGKALLYRVLPEPVKLTLTGGADAQGDIVATELPSIPGTSIGPLDVALASQARWTG
ATRAVDSLSIDNATWVMTDNSNVGALRLASDGSVDFQQPAEAGRFKVLTVRPMIHF
GNDWEDRYYRENMYRO

FIGURE 20F
DNA CONSTRUCTS ENCODING PRION CHIMERAS

Construct designated Tpa.p69.mprpf6 (SEQ ID NO:160):

GAATTCGCCACCATGGATGCAATGAAGAGAGGGCTCTGCTGTGTGCTGCTGCTGT
GTGGAGCAGTCTTCGTTTCGCCCAGCGCTAGCATGAACATGTCTCTGTCACGCAT
TGTCAAGGCGGCGCCCCTGCGCCGCACCACGCTGGCCATGGCGCTGGGCGCGCT
GGGCGCCGCCCCGGCGGCGCATGCCGACTGGAACAACCAGTCCATCGTCAAGAC
CGGTGAGCGCCAGCATGGCATCCATATCCAGGGCTCCGACCCGGGCGGCGTACG
GACCGCCAGCGGAACCACCATCAAGGTAAGCGGCCGTCAGGCCCAGGGCATCCT
GCTAGAAAATCCCGCGGCCGAGCTGCAGTTCCGGAACGGCAGTGTCACGTCGTC
GGGACAGTTGTCCGACGATGGCATCCGGCGCTTTCTGGGCACCGTCACCGTCAAG
GCCGGCAAGCTGGTCGCCGATCACGCCACGCTGGCCAACGTTGGCGACACCTGG
GACGACGACGGCATCGCGCTCTATGTGGCCGGCGAACAGGCCCAGGCCAGCATC
GCCGACAGCACCCTGCAGGGCGCTGGCGGCGTGCAGATCGAGCGCGGCGCCAAT
GTCACGGTCCAACGCAGCGCCATCGTCGACGGGGGCTTGCATATCGGCGCCCTGC
AGTCATTGCAGCCGGAAGACCTTCCGCCCAGCCGGGTGGTGCTGCGCGACACCA
ACGTGACCGCCGTGCCCGCCAGCGGCGCGCCCGCGGCGGTGTCTGTGTTGGGGG
CCAGTGAGCTTACGCTCGACGGCGGGCACATCACCGGCGGGCGGGCAGCGGGGG
TGGCGGCCATGCAAGGGGCGGTCGTGCATCTGCAGCGCGCGACGATACGGCGCG
GGGACGCGCCTGCCGGCGGTGCGGTTCCCGGCGGTGCGGTTCCCGGTGGTGCGG
TTCCCGGCGGCTTCGGTCCCGGCGGCTTCGGTCCCGTCCTCGACGGCTGGTATGG
CGTGGACGTATCGGGCTCCAGCGTGGAGCTCGCCCAGTCGATCGTCGAGGCGCC
GGAGCTGGGCGCCGCAATCCGGGTGGGCCGCGGCGCCAGGGTGACGGTGTCGGG
CGGCAGCTTGTCCGCACCGCACGGCAATGTCATCGAGACCGGCGGCGCGCGTCG
CTTTGCGCCTCAAGCCGCGCCCCTGTCGATCACCTTGCAGGCCGGCGCGCATGCC
CAGGGGAAAGCGCTGCTGTACCGGGTCCTGCCGGAGCCCGTGAAGCTGACGCTG
ACCGGGGGCGCCGATGCGCAGGGCGACATCGTCGCGACGGAGCTGCCCTCCATT
CCCGGCACGTCGATCGGGCCGCTCGACGTGGCGCTGGCCAGCCAGGCCCGATGG
ACGGGCGCTACCCGCGCGGTCGACTCGCTGTCCATCGACAACGCCACCTGGGTCA
TGACGGACAACTCGAACGTCGGTGCGCTACGGCTGGCCAGCGACGGCAGCGTCG
ATTTCCAGCAGCCGGCCGAAGCTGGGCGGTTCAAGGTCCTGACGAGGCCCATGA
TACATTTCGGCAATGACTGGGAGGACCGTTACTATCGTGAAAACATGTATCGTTA
GGATCC

Prion chimera encoded by construct designated Tpa.p69.mprpf6 (SEQ ID NO:131):

MDAMKRGLCCVLLLCGAVFVSPSASMNMSLSRIVKAAPLRRTTLAMALGALGAAP
AAHADWNNQSIVKTGERQHGIHIQGSDPGGVRTASGTTIKVSGRQAQGILLENPAAE
LQFRNGSVTSSGQLSDDGIRRFLGTVTVKAGKLVADHATLANVGDTWDDDGIALYV
AGEQAQASIADSTLQGAGGVQIERGANVTVQRSAIVDGGLHIGALQSLQPEDLPPSR
VVLRDTNVTAVPASGAPAAVSVLGASELTLDGGHITGGRAAGVAAMQGAVVHLQR
ATIRRGDAPAGGAVPGGAVPGGAVPGGFGPGGFGPVLDGWYGVDVSGSSVELAQSI
VEAPELGAAIRVGRGARVTVSGGSLSAPHGNVIETGGARRFAPQAAPLSITLQAGAH
AQGKALLYRVLPEPVKLTLTGGADAQGDIVATELPSIPGTSIGPLDVALASQARWTG
ATRAVDSLSIDNATWVMTDNSNVGALRLASDGSVDFQQPAEAGRFKVLTVRPMIHF
GNDWEDRYYRENMYRO

FIGURE 20G
DNA CONSTRUCTS ENCODING PRION CHIMERAS

Construct designated Tpa.p69.mprpf7 (SEQ ID NO:161):

GAATTCGCCACCATGGATGCAATGAAGAGAGGGCTCTGCTGTGTGCTGCTGCTGT
GTGGAGCAGTCTTCGTTTCGCCCAGCGCTAGCATGAACATGTCTCTGTCACGCAT
TGTCAAGGCGGCGCCCCTGCGCCGCACCACGCTGGCCATGGCGCTGGGCGCGCT
GGGCGCCGCCCCGGCGGCGCATGCCGACTGGAACAACCAGTCCATCGTCAAGAC
CGGTGAGCGCCAGCATGGCATCCATATCCAGGGCTCCGACCCGGGCGGCGTACG
GACCGCCAGCGGAACCACCATCAAGGTAAGCGGCCGTCAGGCCCAGGGCATCCT
GCTAGAAAATCCCGCGGCCGAGCTGCAGTTCCGGAACGGCAGTGTCACGTCGTC
GGGACAGTTGTCCGACGATGGCATCCGGCGCTTTCTGGGCACCGTCACCGTCAAG
GCCGGCAAGCTGGTCGCCGATCACGCCACGCTGGCCAACGTTGGCGACACCTGG
GACGACGACGGCATCGCGCTCTATGTGGCCGGCGAACAGGCCCAGGCCAGCATC
GCCGACAGCACCCTGCAGGGCGCTGGCGGCGTGCAGATCGAGCGCGGCGCCAAT
GTCACGGTCCAACGCAGCGCCATCGTCGACGGGGGCTTGCATATCGGCGCCCTGC
AGTCATTGCAGCCGGAAGACCTTCCGCCCAGCCGGGTGGTGCTGCGCGACACCA
ACGTGACCGCCGTGCCCGCCAGCGGCGCGCCCGCGGCGGTGTCTGTGTTGGGGG
CCAGTGAGCTTACGCTCGACGGCGGGCACATCACCGGCGGGCGGGCAGCGGGGG
TGGCGGCCATGCAAGGGGCGGTCGTGCATCTGCAGCGCGCGACGATACGGCGCG
GGGACGCGCCTGCCGGCGGTGCGGTTCCCGGCGGTGCGGTTCCCGGTGGTGCGG
TTCCCGGCGGCTTCGGTCCCGGCGGCTTCGGTCCCGTCCTCGACGGCTGGTATGG
CGTGGACGTATCGGGCTCCAGCGTGGAGCTCGCCCAGTCGATCGTCGAGGCGCC
GGAGCTGGGCGCCGCAATCCGGGTGGGCCGCGGCGCCAGGGTGACGGTGTCGGG
CGGCAGCTTGTCCGCACCGCACGGCAATGTCATCGAGACCGGCGGCGCGCGTCG
CTTTGCGCCTCAAGCCGCGCCCCTGTCGATCACCTTGCAGGCCGGCGCGCATGCC
CAGGGGAAAGCGCTGCTGTACCGGGTCCTGCCGGAGCCCGTGAAGCTGACGCTG
ACCGGGGGCGCCGATGCGCAGGGCGACATCGTCGCGACGGAGCTGCCCTCCATT
CCCGGCACGTCGATCGGGCCGCTCGACGTGGCGCTGGCCAGCCAGGCCCGATGG
ACGGGCGCTACCCGCGCGGTCGACTCGCTGTCCATCGACAACGCCACCTGGGTCA
TGACGGACAACTCGAACGTCGGTGCGCTACGGCTGGCCAGCGACGGCAGCGTCG
ATTTCCAGCAGCCGGCCGAAGCTGGGCGGTTCAAGGTCCTGAGGCCCATGATAC
ATTTCGGCAATGACTGGGAGGACCGTTACTATCGTGAAAACATGTATCGTTAGGA
TCC

Prion chimera encoded by construct designated Tpa.p69.mprpf7 (SEQ ID NO:132):

MDAMKRGLCCVLLLCGAVFVSPSASMNMSLSRIVKAAPLRRTTLAMALGALGAAP
AAHADWNNQSIVKTGERQHGIHIQGSDPGGVRTASGTTIKVSGRQAQGILLENPAAE
LQFRNGSVTSSGQLSDDGIRRFLGTVTVKAGKLVADHATLANVGDTWDDDGIALYV
AGEQAQASIADSTLQGAGGVQIERGANVTVQRSAIVDGGLHIGALQSLQPEDLPPSR
VVLRDTNVTAVPASGAPAAVSVLGASELTLDGGHITGGRAAGVAAMQGAVVHLQR
ATIRRGDAPAGGAVPGGAVPGGAVPGGFGPGGFGPVLDGWYGVDVSGSSVELAQSI
VEAPELGAAIRVGRGARVTVSGGSLSAPHGNVIETGGARRFAPQAAPLSITLQAGAH
AQGKALLYRVLPEPVKLTLTGGADAQGDIVATELPSIPGTSIGPLDVALASQARWTG
ATRAVDSLSIDNATWVMTDNSNVGALRLASDGSVDFQQPAEAGRFKVLRPMIHFGN
DWEDRYYRENMYRO

FIGURE 20H
DNA CONSTRUCTS ENCODING PRION CHIMERAS

Construct designated Tpa.p69.mprpf8 (SEQ ID NO:162):

GAATTCGCCACCATGGATGCAATGAAGAGAGGGCTCTGCTGTGTGCTGCTGCTGT
GTGGAGCAGTCTTCGTTTCGCCCAGCGCTAGCATGAACATGTCTCTGTCACGCAT
TGTCAAGGCGGCGCCCCTGCGCCGCACCACGCTGGCCATGGCGCTGGGCGCGCT
GGGCGCCGCCCCGGCGGCGCATGCCGACTGGAACAACCAGTCCATCGTCAAGAC
CGGTGAGCGCCAGCATGGCATCCATATCCAGGGCTCCGACCCGGGCGGCGTACG
GACCGCCAGCGGAACCACCATCAAGGTAAGCGGCCGTCAGGCCCAGGGCATCCT
GCTAGAAAATCCCGCGGCCGAGCTGCAGTTCCGGAACGGCAGTGTCACGTCGTC
GGGACAGTTGTCCGACGATGGCATCCGGCGCTTTCTGGGCACCGTCACCGTCAAG
GCCGGCAAGCTGGTCGCCGATCACGCCACGCTGGCCAACGTTGGCGACACCTGG
GACGACGACGGCATCGCGCTCTATGTGGCCGGCGAACAGGCCCAGGCCAGCATC
GCCGACAGCACCCTGCAGGGCGCTGGCGGCGTGCAGATCGAGCGCGGCGCCAAT
GTCACGGTCCAACGCAGCGCCATCGTCGACGGGGGCTTGCATATCGGCGCCCTGC
AGTCATTGCAGCCGGAAGACCTTCCGCCCAGCCGGGTGGTGCTGCGCGACACCA
ACGTGACCGCCGTGCCCGCCAGCGGCGCGCCCGCGGCGGTGTCTGTGTTGGGGG
CCAGTGAGCTTACGCTCGACGGCGGGCACATCACCGGCGGGCGGGCAGCGGGGG
TGGCGGCCATGCAAGGGGCGGTCGTGCATCTGCAGCGCGCGACGATACGGCGCG
GGGACGCGCCTGCCGGCGGTGCGGTTCCCGGCGGTGCGGTTCCCGGTGGTGCGG
TTCCCGGCGGCTTCGGTCCCGGCGGCTTCGGTCCCGTCCTCGACGGCTGGTATGG
CGTGGACGTATCGGGCTCCAGCGTGGAGCTCGCCCAGTCGATCGTCGAGGCGCC
GGAGCTGGGCGCCGCAATCCGGGTGGGCCGCGGCGCCAGGGTGACGGTGTCGGG
CGGCAGCTTGTCCGCACCGCACGGCAATGTCATCGAGACCGGCGGCGCGCGTCG
CTTTGCGCCTCAAGCCGCGCCCCTGTCGATCACCTTGCAGGCCGGCGCGCATGCC
CAGGGGAAAGCGCTGCTGTACCGGGTCCTGCCGGAGCCCGTGAAGCTGACGCTG
ACCGGGGGCGCCGATGCGCAGGGCGACATCGTCGCGACGGAGCTGCCCTCCATT
CCCGGCACGTCGATCGGGCCGCTCGACGTGGCGCTGGCCAGCCAGGCCCGATGG
ACGGGCGCTACCCGCGCGGTCGACTCGCTGTCCATCGACAACGCCACCTGGGTCA
TGACGGACAACTCGAACGTCGGTGCGCTACGGCTGGCCAGCGACGGCAGCGTCG
ATTTCCAGCAGCCGGCCGAAGCTGGGCGGTTCAAGGTCAGGCCCATGATACATTT
CGGCAATGACTGGGAGGACCGTTACTATCGTGAAAACATGTATCGTTAGGATCC

Prion chimera encoded by construct designated Tpa.p69.mprpf8 (SEQ ID NO:133):

MDAMKRGLCCVLLLCGAVFVSPSASMNMSLSRIVKAAPLRRTTLAMALGALGAAP
AAHADWNNQSIVKTGERQHGIHIQGSDPGGVRTASGTTIKVSGRQAQGILLENPAAE
LQFRNGSVTSSGQLSDDGIRRFLGTVTVKAGKLVADHATLANVGDTWDDDGIALYV
AGEQAQASIADSTLQGAGGVQIERGANVTVQRSAIVDGGLHIGALQSLQPEDLPPSR
VVLRDTNVTAVPASGAPAAVSVLGASELTLDGGHITGGRAAGVAAMQGAVVHLQR
ATIRRGDAPAGGAVPGGAVPGGAVPGGFGPGGFGPVLDGWYGVDVSGSSVELAQSI
VEAPELGAAIRVGRGARVTVSGGSLSAPHGNVIETGGARRFAPQAAPLSITLQAGAH
AQGKALLYRVLPEPVKLTLTGGADAQGDIVATELPSIPGTSIGPLDVALASQARWTG
ATRAVDSLSIDNATWVMTDNSNVGALRLASDGSVDFQQPAEAGRFKVRPMIHFGND
WEDRYYRENMYRO

FIGURE 21A
DNA CONSTRUCTS ENCODING PRION CHIMERAS

Construct designated Tpa.mprpf1.69 (SEQ ID NO:163):

GAATTCGCCACCATGGATGCAATGAAGAGAGGGCTCTGCTGTGTGCTGCTGCTGT
GTGGAGCAGTCTTCGTTTCGCCCAGCGCTAGCAGGCCCATGATACATTTCGGCAA
TGACTGGGAGGACCGTTACTATCGTGAAAACATGTATCGTGACTGGAACAACCA
GTCCATCGTCAAGACCGGTGAGCGCCAGCATGGCATCCATATCCAGGGCTCCGA
CCCGGGCGGCGTACGGACCGCCAGCGGAACCACCATCAAGGTAAGCGGCCGTCA
GGCCCAGGGCATCCTGCTAGAAAATCCCGCGGCCGAGCTGCAGTTCCGGAACGG
CAGTGTCACGTCGTCGGGACAGTTGTCCGACGATGGCATCCGGCGCTTTCTGGGC
ACCGTCACCGTCAAGGCCGGCAAGCTGGTCGCCGATCACGCCACGCTGGCCAAC
GTTGGCGACACCTGGGACGACGACGGCATCGCGCTCTATGTGGCCGGCGAACAG
GCCCAGGCCAGCATCGCCGACAGCACCCTGCAGGGCGCTGGCGGCGTGCAGATC
GAGCGCGGCGCCAATGTCACGGTCCAACGCAGCGCCATCGTCGACGGGGGCTTG
CATATCGGCGCCCTGCAGTCATTGCAGCCGGAAGACCTTCCGCCCAGCCGGGTGG
TGCTGCGCGACACCAACGTGACCGCCGTGCCCGCCAGCGGCGCGCCCGCGGCGG
TGTCTGTGTTGGGGGCCAGTGAGCTTACGCTCGACGGCGGGCACATCACCGGCG
GGCGGGCAGCGGGGGTGGCGGCCATGCAAGGGGCGGTCGTGCATCTGCAGCGCG
CGACGATACGGCGCGGGGACGCGCCTGCCGGCGGTGCGGTTCCCGGCGGTGCGG
TTCCCGGTGGTGCGGTTCCCGGCGGCTTCGGTCCCGGCGGCTTCGGTCCCGTCCT
CGACGGCTGGTATGGCGTGGACGTATCGGGCTCCAGCGTGGAGCTCGCCCAGTC
GATCGTCGAGGCGCCGGAGCTGGGCGCCGCAATCCGGGTGGGCCGCGGCGCCAG
GGTGACGGTGTCGGGCGGCAGCTTGTCCGCACCGCACGGCAATGTCATCGAGAC
CGGCGGCGCGCGTCGCTTTGCGCCTCAAGCCGCGCCCCTGTCGATCACCTTGCAG
GCCGGCGCGCATGCCCAGGGGAAAGCGCTGCTGTACCGGGTCCTGCCGGAGCCC
GTGAAGCTGACGCTGACCGGGGGCGCCGATGCGCAGGGCGACATCGTCGCGACG
GAGCTGCCCTCCATTCCCGGCACGTCGATCGGGCCGCTCGACGTGGCGCTGGCCA
GCCAGGCCCGATGGACGGGCGCTACCCGCGCGGTCGACTCGCTGTCCATCGACA
ACGCCACCTGGGTCATGACGGACAACTCGAACGTCGGTGCGCTACGGCTGGCCA
GCGACGGCAGCGTCGATTTCCAGCAGCCGGCCGAAGCTGGGCGGTTCAAGGTCC
TGACGGTCAATACGCTGGCGGGTTCGGGGCTGTTCCGCATGAATGTCTTCGCGGA
CCTGGGGCTGAGCGACAAGCTGGTCGTCATGCAGGACGCCAGCGGCCAGCACAG
GCTGTGGGTCCGCAACAGCGGCAGCGAGCCGGCCAGCGCCAACACCCTGCTGCT
GGTGCAGACGCCACTAGGCAGCGCGGCGACCTTTACCCTTGCCAACAAGGACGG
CAAGGTCGATATCGGTACCTATCGCTATCGATTGGCCGCCAACGGCAATGGGCA
GTGGAGCCTGGTGGGCGCGAAGGCGCCGCCGTAGGATCC

Prion chimera encoded by construct designated Tpa.mprpf1.69 (SEQ ID NO:134):

MDAMKRGLCCVLLLCGAVFVSPSASRPMIHFGNDWEDRYYRENMYRDWNNQSIVK
TGERQHGIHIQGSDPGGVRTASGTTIKVSGRQAQGILLENPAAELQFRNGSVTSSGQL
SDDGIRRFLGTVTVKAGKLVADHATLANVGDTWDDDGIALYVAGEQAQASIADSTL
QGAGGVQIERGANVTVQRSAIVDGGLHIGALQSLQPEDLPPSRVVLRDTNVTAVPAS
GAPAAVSVLGASELTLDGGHITGGRAAGVAAMQGAVVHLQRATIRRGDAPAGGAV
PGGAVPGGAVPGGFGPGGFGPVLDGWYGVDVSGSSVELAQSIVEAPELGAAIRVGR
GARVTVSGGSLSAPHGNVIETGGARRFAPQAAPLSITLQAGAHAQGKALLYRVLPEP
VKLTLTGGADAQGDIVATELPSIPGTSIGPLDVALASQARWTGATRAVDSLSIDNAT
WVMTDNSNVGALRLASDGSVDFQQPAEAGRFKVLTVNTLAGSGLFRMNVFADLGL
SDKLVVMQDASGQHRLWVRNSGSEPASANTLLLVQTPLGSAATFTLANKDGKVDIG
TYRYRLAANGNGQWSLVGAKAPPO

FIGURE 21B
DNA CONSTRUCTS ENCODING PRION CHIMERAS

Construct designated Tpa.mprpf2.69 (SEQ ID NO:164):

GAATTCGCCACCATGGATGCAATGAAGAGAGGGCTCTGCTGTGTGCTGCTGCTGT
GTGGAGCAGTCTTCGTTTCGCCCAGCGCTAGCAGGCCCATGATACATTTCGGCAA
TGACTGGGAGGACCGTTACTATCGTGAAAACATGTATCGTTGGAACAACCAGTCC
ATCGTCAAGACCGGTGAGCGCCAGCATGGCATCCATATCCAGGGCTCCGACCCG
GGCGGCGTACGGACCGCCAGCGGAACCACCATCAAGGTAAGCGGCCGTCAGGCC
CAGGGCATCCTGCTAGAAAATCCCGCGGCCGAGCTGCAGTTCCGGAACGGCAGT
GTCACGTCGTCGGGACAGTTGTCCGACGATGGCATCCGGCGCTTTCTGGGCACCG
TCACCGTCAAGGCCGGCAAGCTGGTCGCCGATCACGCCACGCTGGCCAACGTTG
GCGACACCTGGGACGACGACGGCATCGCGCTCTATGTGGCCGGCGAACAGGCCC
AGGCCAGCATCGCCGACAGCACCCTGCAGGGCGCTGGCGGCGTGCAGATCGAGC
GCGGCGCCAATGTCACGGTCCAACGCAGCGCCATCGTCGACGGGGGCTTGCATA
TCGGCGCCCTGCAGTCATTGCAGCCGGAAGACCTTCCGCCCAGCCGGGTGGTGCT
GCGCGACACCAACGTGACCGCCGTGCCCGCCAGCGGCGCGCCCGCGGCGGTGTC
TGTGTTGGGGGCCAGTGAGCTTACGCTCGACGGCGGGCACATCACCGGCGGGCG
GGCAGCGGGGGTGGCGGCCATGCAAGGGGCGGTCGTGCATCTGCAGCGCGCGAC
GATACGGCGCGGGGACGCGCCTGCCGGCGGTGCGGTTCCCGGCGGTGCGGTTCC
CGGTGGTGCGGTTCCCGGCGGCTTCGGTCCCGGCGGCTTCGGTCCCGTCCTCGAC
GGCTGGTATGGCGTGGACGTATCGGGCTCCAGCGTGGAGCTCGCCCAGTCGATC
GTCGAGGCGCCGGAGCTGGGCGCCGCAATCCGGGTGGGCCGCGGCGCCAGGGTG
ACGGTGTCGGGCGGCAGCTTGTCCGCACCGCACGGCAATGTCATCGAGACCGGC
GGCGCGCGTCGCTTTGCGCCTCAAGCCGCGCCCCTGTCGATCACCTTGCAGGCCG
GCGCGCATGCCCAGGGGAAAGCGCTGCTGTACCGGGTCCTGCCGGAGCCCGTGA
AGCTGACGCTGACCGGGGGCGCCGATGCGCAGGGCGACATCGTCGCGACGGAGC
TGCCCTCCATTCCCGGCACGTCGATCGGGCCGCTCGACGTGGCGCTGGCCAGCCA
GGCCCGATGGACGGGCGCTACCCGCGCGGTCGACTCGCTGTCCATCGACAACGC
CACCTGGGTCATGACGGACAACTCGAACGTCGGTGCGCTACGGCTGGCCAGCGA
CGGCAGCGTCGATTTCCAGCAGCCGGCCGAAGCTGGGCGGTTCAAGGTCCTGAC
GGTCAATACGCTGGCGGGTTCGGGGCTGTTCCGCATGAATGTCTTCGCGGACCTG
GGGCTGAGCGACAAGCTGGTCGTCATGCAGGACGCCAGCGGCCAGCACAGGCTG
TGGGTCCGCAACAGCGGCAGCGAGCCGGCCAGCGCCAACACCCTGCTGCTGGTG
CAGACGCCACTAGGCAGCGCGGCGACCTTTACCCTTGCCAACAAGGACGGCAAG
GTCGATATCGGTACCTATCGCTATCGATTGGCCGCCAACGGCAATGGGCAGTGGA
GCCTGGTGGGCGCGAAGGCGCCGCCGTAGGATCC

Prion chimera encoded by construct designated Tpa.mprpf2.69 (SEQ ID NO:135):

MDAMKRGLCCVLLLCGAVFVSPSASRPMIHFGNDWEDRYYRENMYRWNNQSIVKT
GERQHGIHIQGSDPGGVRTASGTTIKVSGRQAQGILLENPAAELQFRNGSVTSSGQLS
DDGIRRFLGTVTVKAGKLVADHATLANVGDTWDDDGIALYVAGEQAQASIADSTL
QGAGGVQIERGANVTVQRSAIVDGGLHIGALQSLQPEDLPPSRVVLRDTNVTAVPAS
GAPAAVSVLGASELTLDGGHITGGRAAGVAAMQGAVVHLQRATIRRGDAPAGGAV
PGGAVPGGAVPGGFGPGGFGPVLDGWYGVDVSGSSVELAQSIVEAPELGAAIRVGR
GARVTVSGGSLSAPHGNVIETGGARRFAPQAAPLSITLQAGAHAQGKALLYRVLPEP
VKLTLTGGADAQGDIVATELPSIPGTSIGPLDVALASQARWTGATRAVDSLSIDNAT
WVMTDNSNVGALRLASDGSVDFQQPAEAGRFKVLTVNTLAGSGLFRMNVFADLGL
SDKLVVMQDASGQHRLWVRNSGSEPASANTLLLVQTPLGSAATFTLANKDGKVDIG
TYRYRLAANGNGQWSLVGAKAPPO

FIGURE 21C
DNA CONSTRUCTS ENCODING PRION CHIMERAS

Construct designated Tpa.mprpf3.69 (SEQ ID NO:165):

GAATTCGCCACCATGGATGCAATGAAGAGAGGGCTCTGCTGTGTGCTGCTGCTGT
GTGGAGCAGTCTTCGTTTCGCCCAGCGCTAGCAGGCCCATGATACATTTCGGCAA
TGACTGGGAGGACCGTTACTATCGTGAAAACATGTATCGTAACAACCAGTCCATC
GTCAAGACCGGTGAGCGCCAGCATGGCATCCATATCCAGGGCTCCGACCCGGGC
GGCGTACGGACCGCCAGCGGAACCACCATCAAGGTAAGCGGCCGTCAGGCCCAG
GGCATCCTGCTAGAAAATCCCGCGGCCGAGCTGCAGTTCCGGAACGGCAGTGTC
ACGTCGTCGGGACAGTTGTCCGACGATGGCATCCGGCGCTTTCTGGGCACCGTCA
CCGTCAAGGCCGGCAAGCTGGTCGCCGATCACGCCACGCTGGCCAACGTTGGCG
ACACCTGGGACGACGACGGCATCGCGCTCTATGTGGCCGGCGAACAGGCCCAGG
CCAGCATCGCCGACAGCACCCTGCAGGGCGCTGGCGGCGTGCAGATCGAGCGCG
GCGCCAATGTCACGGTCCAACGCAGCGCCATCGTCGACGGGGGCTTGCATATCG
GCGCCCTGCAGTCATTGCAGCCGGAAGACCTTCCGCCCAGCCGGGTGGTGCTGCG
CGACACCAACGTGACCGCCGTGCCCGCCAGCGGCGCGCCCGCGGCGGTGTCTGT
GTTGGGGGCCAGTGAGCTTACGCTCGACGGCGGGCACATCACCGGCGGGCGGGC
AGCGGGGGTGGCGGCCATGCAAGGGGCGGTCGTGCATCTGCAGCGCGCGACGAT
ACGGCGCGGGGACGCGCCTGCCGGCGGTGCGGTTCCCGGCGGTGCGGTTCCCGG
TGGTGCGGTTCCCGGCGGCTTCGGTCCCGGCGGCTTCGGTCCCGTCCTCGACGGC
TGGTATGGCGTGGACGTATCGGGCTCCAGCGTGGAGCTCGCCCAGTCGATCGTCG
AGGCGCCGGAGCTGGGCGCCGCAATCCGGGTGGGCCGCGGCGCCAGGGTGACGG
TGTCGGGCGGCAGCTTGTCCGCACCGCACGGCAATGTCATCGAGACCGGCGGCG
CGCGTCGCTTTGCGCCTCAAGCCGCGCCCCTGTCGATCACCTTGCAGGCCGGCGC
GCATGCCCAGGGGAAAGCGCTGCTGTACCGGGTCCTGCCGGAGCCCGTGAAGCT
GACGCTGACCGGGGGCGCCGATGCGCAGGGCGACATCGTCGCGACGGAGCTGCC
CTCCATTCCCGGCACGTCGATCGGGCCGCTCGACGTGGCGCTGGCCAGCCAGGCC
CGATGGACGGGCGCTACCCGCGCGGTCGACTCGCTGTCCATCGACAACGCCACCT
GGGTCATGACGGACAACTCGAACGTCGGTGCGCTACGGCTGGCCAGCGACGGCA
GCGTCGATTTCCAGCAGCCGGCCGAAGCTGGGCGGTTCAAGGTCCTGACGGTCA
ATACGCTGGCGGGTTCGGGGCTGTTCCGCATGAATGTCTTCGCGGACCTGGGGCT
GAGCGACAAGCTGGTCGTCATGCAGGACGCCAGCGGCCAGCACAGGCTGTGGGT
CCGCAACAGCGGCAGCGAGCCGGCCAGCGCCAACACCCTGCTGCTGGTGCAGAC
GCCACTAGGCAGCGCGGCGACCTTTACCCTTGCCAACAAGGACGGCAAGGTCGA
TATCGGTACCTATCGCTATCGATTGGCCGCCAACGGCAATGGGCAGTGGAGCCTG
GTGGGCGCGAAGGCGCCGCCGTAGGATCC

Prion chimera encoded by construct designated Tpa.mprpf3.69 (SEQ ID NO:136):

MDAMKRGLCCVLLLCGAVFVSPSASRPMIHFGNDWEDRYYRENMYRNNQSIVKTG
ERQHGIHIQGSDPGGVRTASGTTIKVSGRQAQGILLENPAAELQFRNGSVTSSGQLSD
DGIRRFLGTVTVKAGKLVADHATLANVGDTWDDDGIALYVAGEQAQASIADSTLQ
GAGGVQIERGANVTVQRSAIVDGGLHIGALQSLQPEDLPPSRVVLRDTNVTAVPASG
APAAVSVLGASELTLDGGHITGGRAAGVAAMQGAVVHLQRATIRRGDAPAGGAVP
GGAVPGGAVPGGFGPGGFGPVLDGWYGVDVSGSSVELAQSIVEAPELGAAIRVGRG
ARVTVSGGSLSAPHGNVIETGGARRFAPQAAPLSITLQAGAHAQGKALLYRVLPEPV
KLTLTGGADAQGDIVATELPSIPGTSIGPLDVALASQARWTGATRAVDSLSIDNATW
VMTDNSNVGALRLASDGSVDFQQPAEAGRFKVLTVNTLAGSGLFRMNVFADLGLS
DKLVVMQDASGQHRLWVRNSGSEPASANTLLLVQTPLGSAATFTLANKDGKVDIGT
YRYRLAANGNGQWSLVGAKAPPO

FIGURE 21D
DNA CONSTRUCTS ENCODING PRION CHIMERAS

Construct designated Tpa.mprpf4.69 (SEQ ID NO:166):

GAATTCGCCACCATGGATGCAATGAAGAGAGGGCTCTGCTGTGTGCTGCTGCTGT
GTGGAGCAGTCTTCGTTTCGCCCAGCGCTAGCAGGCCCATGATACATTTCGGCAA
TGACTGGGAGGACCGTTACTATCGTGAAAACATGTATCGTAACCAGTCCATCGTC
AAGACCGGTGAGCGCCAGCATGGCATCCATATCCAGGGCTCCGACCCGGGCGGC
GTACGGACCGCCAGCGGAACCACCATCAAGGTAAGCGGCCGTCAGGCCCAGGGC
ATCCTGCTAGAAAATCCCGCGGCCGAGCTGCAGTTCCGGAACGGCAGTGTCACG
TCGTCGGGACAGTTGTCCGACGATGGCATCCGGCGCTTTCTGGGCACCGTCACCG
TCAAGGCCGGCAAGCTGGTCGCCGATCACGCCACGCTGGCCAACGTTGGCGACA
CCTGGGACGACGACGGCATCGCGCTCTATGTGGCCGGCGAACAGGCCCAGGCCA
GCATCGCCGACAGCACCCTGCAGGGCGCTGGCGGCGTGCAGATCGAGCGCGGCG
CCAATGTCACGGTCCAACGCAGCGCCATCGTCGACGGGGGCTTGCATATCGGCG
CCCTGCAGTCATTGCAGCCGGAAGACCTTCCGCCCAGCCGGGTGGTGCTGCGCGA
CACCAACGTGACCGCCGTGCCCGCCAGCGGCGCGCCCGCGGCGGTGTCTGTGTTG
GGGGCCAGTGAGCTTACGCTCGACGGCGGGCACATCACCGGCGGGCGGGCAGCG
GGGGTGGCGGCCATGCAAGGGGCGGTCGTGCATCTGCAGCGCGCGACGATACGG
CGCGGGGACGCGCCTGCCGGCGGTGCGGTTCCCGGCGGTGCGGTTCCCGGTGGT
GCGGTTCCCGGCGGCTTCGGTCCCGGCGGCTTCGGTCCCGTCCTCGACGGCTGGT
ATGGCGTGGACGTATCGGGCTCCAGCGTGGAGCTCGCCCAGTCGATCGTCGAGG
CGCCGGAGCTGGGCGCCGCAATCCGGGTGGGCCGCGGCGCCAGGGTGACGGTGT
CGGGCGGCAGCTTGTCCGCACCGCACGGCAATGTCATCGAGACCGGCGGCGCGC
GTCGCTTTGCGCCTCAAGCCGCGCCCCTGTCGATCACCTTGCAGGCCGGCGCGCA
TGCCCAGGGGAAAGCGCTGCTGTACCGGGTCCTGCCGGAGCCCGTGAAGCTGAC
GCTGACCGGGGGCGCCGATGCGCAGGGCGACATCGTCGCGACGGAGCTGCCCTC
CATTCCCGGCACGTCGATCGGGCCGCTCGACGTGGCGCTGGCCAGCCAGGCCCG
ATGGACGGGCGCTACCCGCGCGGTCGACTCGCTGTCCATCGACAACGCCACCTG
GGTCATGACGGACAACTCGAACGTCGGTGCGCTACGGCTGGCCAGCGACGGCAG
CGTCGATTTCCAGCAGCCGGCCGAAGCTGGGCGGTTCAAGGTCCTGACGGTCAAT
ACGCTGGCGGGTTCGGGGCTGTTCCGCATGAATGTCTTCGCGGACCTGGGGCTGA
GCGACAAGCTGGTCGTCATGCAGGACGCCAGCGGCCAGCACAGGCTGTGGGTCC
GCAACAGCGGCAGCGAGCCGGCCAGCGCCAACACCCTGCTGCTGGTGCAGACGC
CACTAGGCAGCGCGGCGACCTTTACCCTTGCCAACAAGGACGGCAAGGTCGATA
TCGGTACCTATCGCTATCGATTGGCCGCCAACGGCAATGGGCAGTGGAGCCTGGT
GGGCGCGAAGGCGCCGCCGTAGGATCC

Prion chimera encoded by construct designated Tpa.mprpf469 (SEQ ID NO:137):

MDAMKRGLCCVLLLCGAVFVSPSASRPMIHFGNDWEDRYYRENMYRNQSIVKTGE
RQHGIHIQGSDPGGVRTASGTTIKVSGRQAQGILLENPAAELQFRNGSVTSSGQLSDD
GIRRFLGTVTVKAGKLVADHATLANVGDTWDDDGIALYVAGEQAQASIADSTLQG
AGGVQIERGANVTVQRSAIVDGGLHIGALQSLQPEDLPPSRVVLRDTNVTAVPASGA
PAAVSVLGASELTLDGGHITGGRAAGVAAMQGAVVHLQRATIRRGDAPAGGAVPG
GAVPGGAVPGGFGPGGFGPVLDGWYGVDVSGSSVELAQSIVEAPELGAAIRVGRGA
RVTVSGGSLSAPHGNVIETGGARRFAPQAAPLSITLQAGAHAQGKALLYRVLPEPVK
LTLTGGADAQGDIVATELPSIPGTSIGPLDVALASQARWTGATRAVDSLSIDNATWV
MTDNSNVGALRLASDGSVDFQQPAEAGRFKVLTVNTLAGSGLFRMNVFADLGLSD
KLVVMQDASGQHRLWVRNSGSEPASANTLLLVQTPLGSAATFTLANKDGKVDIGTY
RYRLAANGNGQWSLVGAKAPPO

FIGURE 21E
DNA CONSTRUCTS ENCODING PRION CHIMERAS

Construct designated Tpa.mprpf5.69 (SEQ ID NO:167):

GAATTCGCCACCATGGATGCAATGAAGAGAGGGCTCTGCTGTGTGCTGCTGCTGT
GTGGAGCAGTCTTCGTTTCGCCCAGCGCTAGCAGGCCCATGATACATTTCGGCAA
TGACTGGGAGGACCGTTACTATCGTGAAAACATGTATCGTCAGTCCATCGTCAAG
ACCGGTGAGCGCCAGCATGGCATCCATATCCAGGGCTCCGACCCGGGCGGCGTA
CGGACCGCCAGCGGAACCACCATCAAGGTAAGCGGCCGTCAGGCCCAGGGCATC
CTGCTAGAAAATCCCGCGGCCGAGCTGCAGTTCCGGAACGGCAGTGTCACGTCG
TCGGGACAGTTGTCCGACGATGGCATCCGGCGCTTTCTGGGCACCGTCACCGTCA
AGGCCGGCAAGCTGGTCGCCGATCACGCCACGCTGGCCAACGTTGGCGACACCT
GGGACGACGACGGCATCGCGCTCTATGTGGCCGGCGAACAGGCCCAGGCCAGCA
TCGCCGACAGCACCCTGCAGGGCGCTGGCGGCGTGCAGATCGAGCGCGGCGCCA
ATGTCACGGTCCAACGCAGCGCCATCGTCGACGGGGGCTTGCATATCGGCGCCCT
GCAGTCATTGCAGCCGGAAGACCTTCCGCCCAGCCGGGTGGTGCTGCGCGACAC
CAACGTGACCGCCGTGCCCGCCAGCGGCGCGCCCGCGGCGGTGTCTGTGTTGGG
GGCCAGTGAGCTTACGCTCGACGGCGGGCACATCACCGGCGGGCGGGCAGCGGG
GGTGGCGGCCATGCAAGGGGCGGTCGTGCATCTGCAGCGCGCGACGATACGGCG
CGGGGACGCGCCTGCCGGCGGTGCGGTTCCCGGCGGTGCGGTTCCCGGTGGTGC
GGTTCCCGGCGGCTTCGGTCCCGGCGGCTTCGGTCCCGTCCTCGACGGCTGGTAT
GGCGTGGACGTATCGGGCTCCAGCGTGGAGCTCGCCCAGTCGATCGTCGAGGCG
CCGGAGCTGGGCGCCGCAATCCGGGTGGGCCGCGGCGCCAGGGTGACGGTGTCG
GGCGGCAGCTTGTCCGCACCGCACGGCAATGTCATCGAGACCGGCGGCGCGCGT
CGCTTTGCGCCTCAAGCCGCGCCCCTGTCGATCACCTTGCAGGCCGGCGCGCATG
CCCAGGGGAAAGCGCTGCTGTACCGGGTCCTGCCGGAGCCCGTGAAGCTGACGC
TGACCGGGGGCGCCGATGCGCAGGGCGACATCGTCGCGACGGAGCTGCCCTCCA
TTCCCGGCACGTCGATCGGGCCGCTCGACGTGGCGCTGGCCAGCCAGGCCCGAT
GGACGGGCGCTACCCGCGCGGTCGACTCGCTGTCCATCGACAACGCCACCTGGG
TCATGACGGACAACTCGAACGTCGGTGCGCTACGGCTGGCCAGCGACGGCAGCG
TCGATTTCCAGCAGCCGGCCGAAGCTGGGCGGTTCAAGGTCCTGACGGTCAATAC
GCTGGCGGGTTCGGGGCTGTTCCGCATGAATGTCTTCGCGGACCTGGGGCTGAGC
GACAAGCTGGTCGTCATGCAGGACGCCAGCGGCCAGCACAGGCTGTGGGTCCGC
AACAGCGGCAGCGAGCCGGCCAGCGCCAACACCCTGCTGCTGGTGCAGACGCCA
CTAGGCAGCGCGGCGACCTTTACCCTTGCCAACAAGGACGGCAAGGTCGATATC
GGTACCTATCGCTATCGATTGGCCGCCAACGGCAATGGGCAGTGGAGCCTGGTG
GGCGCGAAGGCGCCGCCGTAGGATCC

Prion chimera encoded by construct designated Tpa.mprpf5.69 (SEQ ID NO:138):

MDAMKRGLCCVLLLCGAVFVSPSASRPMIHFGNDWEDRYYRENMYRQSIVKTGER
QHGIHIQGSDPGGVRTASGTTIKVSGRQAQGILLENPAAELQFRNGSVTSSGQLSDDG
IRRFLGTVTVKAGKLVADHATLANVGDTWDDDGIALYVAGEQAQASIADSTLQGA
GGVQIERGANVTVQRSAIVDGGLHIGALQSLQPEDLPPSRVVLRDTNVTAVPASGAP
AAVSVLGASELTLDGGHITGGRAAGVAAMQGAVVHLQRATIRRGDAPAGGAVPGG
AVPGGAVPGGFGPGGFGPVLDGWYGVDVSGSSVELAQSIVEAPELGAAIRVGRGAR
VTVSGGSLSAPHGNVIETGGARRFAPQAAPLSITLQAGAHAQGKALLYRVLPEPVKL
TLTGGADAQGDIVATELPSIPGTSIGPLDVALASQARWTGATRAVDSLSIDNATWVM
TDNSNVGALRLASDGSVDFQQPAEAGRFKVLTVNTLAGSGLFRMNVFADLGLSDKL
VVMQDASGQHRLWVRNSGSEPASANTLLLVQTPLGSAATFTLANKDGKVDIGTYRY
RLAANGNGQWSLVGAKAPPO

FIGURE 21F
DNA CONSTRUCTS ENCODING PRION CHIMERAS

Construct designated Tpa.mprpf6.69 (SEQ ID NO:168):

GAATTCGCCACCATGGATGCAATGAAGAGAGGGCTCTGCTGTGTGCTGCTGCTGT
GTGGAGCAGTCTTCGTTTCGCCCAGCGCTAGCAGGCCCATGATACATTTCGGCAA
TGACTGGGAGGACCGTTACTATCGTGAAAACATGTATCGTTCCATCGTCAAGACC
GGTGAGCGCCAGCATGGCATCCATATCCAGGGCTCCGACCCGGGCGGCGTACGG
ACCGCCAGCGGAACCACCATCAAGGTAAGCGGCCGTCAGGCCCAGGGCATCCTG
CTAGAAAATCCCGCGGCCGAGCTGCAGTTCCGGAACGGCAGTGTCACGTCGTCG
GGACAGTTGTCCGACGATGGCATCCGGCGCTTTCTGGGCACCGTCACCGTCAAGG
CCGGCAAGCTGGTCGCCGATCACGCCACGCTGGCCAACGTTGGCGACACCTGGG
ACGACGACGGCATCGCGCTCTATGTGGCCGGCGAACAGGCCCAGGCCAGCATCG
CCGACAGCACCCTGCAGGGCGCTGGCGGCGTGCAGATCGAGCGCGGCGCCAATG
TCACGGTCCAACGCAGCGCCATCGTCGACGGGGGCTTGCATATCGGCGCCCTGCA
GTCATTGCAGCCGGAAGACCTTCCGCCCAGCCGGGTGGTGCTGCGCGACACCAA
CGTGACCGCCGTGCCCGCCAGCGGCGCGCCCGCGGCGGTGTCTGTGTTGGGGGC
CAGTGAGCTTACGCTCGACGGCGGGCACATCACCGGCGGGCGGGCAGCGGGGGT
GGCGGCCATGCAAGGGGCGGTCGTGCATCTGCAGCGCGCGACGATACGGCGCGG
GGACGCGCCTGCCGGCGGTGCGGTTCCCGGCGGTGCGGTTCCCGGTGGTGCGGTT
CCCGGCGGCTTCGGTCCCGGCGGCTTCGGTCCCGTCCTCGACGGCTGGTATGGCG
TGGACGTATCGGGCTCCAGCGTGGAGCTCGCCCAGTCGATCGTCGAGGCGCCGG
AGCTGGGCGCCGCAATCCGGGTGGGCCGCGGCGCCAGGGTGACGGTGTCGGGCG
GCAGCTTGTCCGCACCGCACGGCAATGTCATCGAGACCGGCGGCGCGCGTCGCTT
TGCGCCTCAAGCCGCGCCCCTGTCGATCACCTTGCAGGCCGGCGCGCATGCCCAG
GGGAAAGCGCTGCTGTACCGGGTCCTGCCGGAGCCCGTGAAGCTGACGCTGACC
GGGGGCGCCGATGCGCAGGGCGACATCGTCGCGACGGAGCTGCCCTCCATTCCC
GGCACGTCGATCGGGCCGCTCGACGTGGCGCTGGCCAGCCAGGCCCGATGGACG
GGCGCTACCCGCGCGGTCGACTCGCTGTCCATCGACAACGCCACCTGGGTCATGA
CGGACAACTCGAACGTCGGTGCGCTACGGCTGGCCAGCGACGGCAGCGTCGATT
TCCAGCAGCCGGCCGAAGCTGGGCGGTTCAAGGTCCTGACGGTCAATACGCTGG
CGGGTTCGGGGCTGTTCCGCATGAATGTCTTCGCGGACCTGGGGCTGAGCGACAA
GCTGGTCGTCATGCAGGACGCCAGCGGCCAGCACAGGCTGTGGGTCCGCAACAG
CGGCAGCGAGCCGGCCAGCGCCAACACCCTGCTGCTGGTGCAGACGCCACTAGG
CAGCGCGGCGACCTTTACCCTTGCCAACAAGGACGGCAAGGTCGATATCGGTAC
CTATCGCTATCGATTGGCCGCCAACGGCAATGGGCAGTGGAGCCTGGTGGGCGC
GAAGGCGCCGCCGTAGGATCC

Prion chimera encoded by construct designated Tpa.mprpf6.69 (SEQ ID NO:139):

MDAMKRGLCCVLLLCGAVFVSPSASRPMIHFGNDWEDRYYRENMYRSIVKTGERQ
HGIHIQGSDPGGVRTASGTTIKVSGRQAQGILLENPAAELQFRNGSVTSSGQLSDDGI
RRFLGTVTVKAGKLVADHATLANVGDTWDDDGIALYVAGEQAQASIADSTLQGAG
GVQIERGANVTVQRSAIVDGGLHIGALQSLQPEDLPPSRVVLRDTNVTAVPASGAPA
AVSVLGASELTLDGGHITGGRAAGVAAMQGAVVHLQRATIRRGDAPAGGAVPGGA
VPGGAVPGGFGPGGFGPVLDGWYGVDVSGSSVELAQSIVEAPELGAAIRVGRGARV
TVSGGSLSAPHGNVIETGGARRFAPQAAPLSITLQAGAHAQGKALLYRVLPEPVKLT
LTGGADAQGDIVATELPSIPGTSIGPLDVALASQARWTGATRAVDSLSIDNATWVMT
DNSNVGALRLASDGSVDFQQPAEAGRFKVLTVNTLAGSGLFRMNVFADLGLSDKLV
VMQDASGQHRLWVRNSGSEPASANTLLLVQTPLGSAATFTLANKDGKVDIGTYRYR
LAANGNGQWSLVGAKAPPO

FIGURE 21G
DNA CONSTRUCTS ENCODING PRION CHIMERAS

Construct designated Tpa.mprpf7.69 (SEQ ID NO:169):

GAATTCGCCACCATGGATGCAATGAAGAGAGGGCTCTGCTGTGTGCTGCTGCTGT
GTGGAGCAGTCTTCGTTTCGCCCAGCGCTAGCAGGCCCATGATACATTTCGGCAA
TGACTGGGAGGACCGTTACTATCGTGAAAACATGTATCGTATCGTCAAGACCGGT
GAGCGCCAGCATGGCATCCATATCCAGGGCTCCGACCCGGGCGGCGTACGGACC
GCCAGCGGAACCACCATCAAGGTAAGCGGCCGTCAGGCCCAGGGCATCCTGCTA
GAAAATCCCGCGGCCGAGCTGCAGTTCCGGAACGGCAGTGTCACGTCGTCGGGA
CAGTTGTCCGACGATGGCATCCGGCGCTTTCTGGGCACCGTCACCGTCAAGGCCG
GCAAGCTGGTCGCCGATCACGCCACGCTGGCCAACGTTGGCGACACCTGGGACG
ACGACGGCATCGCGCTCTATGTGGCCGGCGAACAGGCCCAGGCCAGCATCGCCG
ACAGCACCCTGCAGGGCGCTGGCGGCGTGCAGATCGAGCGCGGCGCCAATGTCA
CGGTCCAACGCAGCGCCATCGTCGACGGGGGCTTGCATATCGGCGCCCTGCAGTC
ATTGCAGCCGGAAGACCTTCCGCCCAGCCGGGTGGTGCTGCGCGACACCAACGT
GACCGCCGTGCCCGCCAGCGGCGCGCCCGCGGCGGTGTCTGTGTTGGGGGCCAG
TGAGCTTACGCTCGACGGCGGGCACATCACCGGCGGGCGGGCAGCGGGGGTGGC
GGCCATGCAAGGGGCGGTCGTGCATCTGCAGCGCGCGACGATACGGCGCGGGGA
CGCGCCTGCCGGCGGTGCGGTTCCCGGCGGTGCGGTTCCCGGTGGTGCGGTTCCC
GGCGGCTTCGGTCCCGGCGGCTTCGGTCCCGTCCTCGACGGCTGGTATGGCGTGG
ACGTATCGGGCTCCAGCGTGGAGCTCGCCCAGTCGATCGTCGAGGCGCCGGAGC
TGGGCGCCGCAATCCGGGTGGGCCGCGGCGCCAGGGTGACGGTGTCGGGCGGCA
GCTTGTCCGCACCGCACGGCAATGTCATCGAGACCGGCGGCGCGCGTCGCTTTGC
GCCTCAAGCCGCGCCCCTGTCGATCACCTTGCAGGCCGGCGCGCATGCCCAGGG
GAAAGCGCTGCTGTACCGGGTCCTGCCGGAGCCCGTGAAGCTGACGCTGACCGG
GGGCGCCGATGCGCAGGGCGACATCGTCGCGACGGAGCTGCCCTCCATTCCCGG
CACGTCGATCGGGCCGCTCGACGTGGCGCTGGCCAGCCAGGCCCGATGGACGGG
CGCTACCCGCGCGGTCGACTCGCTGTCCATCGACAACGCCACCTGGGTCATGACG
GACAACTCGAACGTCGGTGCGCTACGGCTGGCCAGCGACGGCAGCGTCGATTTC
CAGCAGCCGGCCGAAGCTGGGCGGTTCAAGGTCCTGACGGTCAATACGCTGGCG
GGTTCGGGGCTGTTCCGCATGAATGTCTTCGCGGACCTGGGGCTGAGCGACAAGC
TGGTCGTCATGCAGGACGCCAGCGGCCAGCACAGGCTGTGGGTCCGCAACAGCG
GCAGCGAGCCGGCCAGCGCCAACACCCTGCTGCTGGTGCAGACGCCACTAGGCA
GCGCGGCGACCTTTACCCTTGCCAACAAGGACGGCAAGGTCGATATCGGTACCT
ATCGCTATCGATTGGCCGCCAACGGCAATGGGCAGTGGAGCCTGGTGGGCGCGA
AGGCGCCGCCGTAGGATCC

Prion chimera encoded by construct designated Tpa.mprpf7.69 (SEQ ID NO:140):

MDAMKRGLCCVLLLCGAVFVSPSASRPMIHFGNDWEDRYYRENMYRIVKTGERQH
GIHIQGSDPGGVRTASGTTIKVSGRQAQGILLENPAAELQFRNGSVTSSGQLSDDGIRR
FLGTVTVKAGKLVADHATLANVGDTWDDDGIALYVAGEQAQASIADSTLQGAGGV
QIERGANVTVQRSAIVDGGLHIGALQSLQPEDLPPSRVVLRDTNVTAVPASGAPAAV
SVLGASELTLDGGHITGGRAAGVAAMQGAVVHLQRATIRRGDAPAGGAVPGGAVP
GGAVPGGFGPGGFGPVLDGWYGVDVSGSSVELAQSIVEAPELGAAIRVGRGARVTV
SGGSLSAPHGNVIETGGARRFAPQAAPLSITLQAGAHAQGKALLYRVLPEPVKLTLT
GGADAQGDIVATELPSIPGTSIGPLDVALASQARWTGATRAVDSLSIDNATWVMTDN
SNVGALRLASDGSVDFQQPAEAGRFKVLTVNTLAGSGLFRMNVFADLGLSDKLVV
MQDASGQHRLWVRNSGSEPASANTLLLVQTPLGSAATFTLANKDGKVDIGTYRYRL
AANGNGQWSLVGAKAPPO

FIGURE 21H
DNA CONSTRUCTS ENCODING PRION CHIMERAS

Construct designated Tpa.mprpf8.69 (SEQ ID NO:170):

GAATTCGCCACCATGGATGCAATGAAGAGAGGGCTCTGCTGTGTGCTGCTGCTGT
GTGGAGCAGTCTTCGTTTCGCCCAGCGCTAGCAGGCCCATGATACATTTCGGCAA
TGACTGGGAGGACCGTTACTATCGTGAAAACATGTATCGTGTCAAGACCGGTGA
GCGCCAGCATGGCATCCATATCCAGGGCTCCGACCCGGGCGGCGTACGGACCGC
CAGCGGAACCACCATCAAGGTAAGCGGCCGTCAGGCCCAGGGCATCCTGCTAGA
AAATCCCGCGGCCGAGCTGCAGTTCCGGAACGGCAGTGTCACGTCGTCGGGACA
GTTGTCCGACGATGGCATCCGGCGCTTTCTGGGCACCGTCACCGTCAAGGCCGGC
AAGCTGGTCGCCGATCACGCCACGCTGGCCAACGTTGGCGACACCTGGGACGAC
GACGGCATCGCGCTCTATGTGGCCGGCGAACAGGCCCAGGCCAGCATCGCCGAC
AGCACCCTGCAGGGCGCTGGCGGCGTGCAGATCGAGCGCGGCGCCAATGTCACG
GTCCAACGCAGCGCCATCGTCGACGGGGGCTTGCATATCGGCGCCCTGCAGTCAT
TGCAGCCGGAAGACCTTCCGCCCAGCCGGGTGGTGCTGCGCGACACCAACGTGA
CCGCCGTGCCCGCCAGCGGCGCGCCCGCGGCGGTGTCTGTGTTGGGGGCCAGTG
AGCTTACGCTCGACGGCGGGCACATCACCGGCGGGCGGGCAGCGGGGGTGGCGG
CCATGCAAGGGGCGGTCGTGCATCTGCAGCGCGCGACGATACGGCGCGGGGACG
CGCCTGCCGGCGGTGCGGTTCCCGGCGGTGCGGTTCCCGGTGGTGCGGTTCCCGG
CGGCTTCGGTCCCGGCGGCTTCGGTCCCGTCCTCGACGGCTGGTATGGCGTGGAC
GTATCGGGCTCCAGCGTGGAGCTCGCCCAGTCGATCGTCGAGGCGCCGGAGCTG
GGCGCCGCAATCCGGGTGGGCCGCGGCGCCAGGGTGACGGTGTCGGGCGGCAGC
TTGTCCGCACCGCACGGCAATGTCATCGAGACCGGCGGCGCGCGTCGCTTTGCGC
CTCAAGCCGCGCCCCTGTCGATCACCTTGCAGGCCGGCGCGCATGCCCAGGGGA
AAGCGCTGCTGTACCGGGTCCTGCCGGAGCCCGTGAAGCTGACGCTGACCGGGG
GCGCCGATGCGCAGGGCGACATCGTCGCGACGGAGCTGCCCTCCATTCCCGGCA
CGTCGATCGGGCCGCTCGACGTGGCGCTGGCCAGCCAGGCCCGATGGACGGGCG
CTACCCGCGCGGTCGACTCGCTGTCCATCGACAACGCCACCTGGGTCATGACGGA
CAACTCGAACGTCGGTGCGCTACGGCTGGCCAGCGACGGCAGCGTCGATTTCCA
GCAGCCGGCCGAAGCTGGGCGGTTCAAGGTCCTGACGGTCAATACGCTGGCGGG
TTCGGGGCTGTTCCGCATGAATGTCTTCGCGGACCTGGGGCTGAGCGACAAGCTG
GTCGTCATGCAGGACGCCAGCGGCCAGCACAGGCTGTGGGTCCGCAACAGCGGC
AGCGAGCCGGCCAGCGCCAACACCCTGCTGCTGGTGCAGACGCCACTAGGCAGC
GCGGCGACCTTTACCCTTGCCAACAAGGACGGCAAGGTCGATATCGGTACCTATC
GCTATCGATTGGCCGCCAACGGCAATGGGCAGTGGAGCCTGGTGGGCGCGAAGG
CGCCGCCGTAGGATCC

Prion chimera encoded by construct designated Tpa.mprpf869 (SEQ ID NO:141):

MDAMKRGLCCVLLLCGAVFVSPSASRPMIHFGNDWEDRYYRENMYRVKTGERQH
GIHIQGSDPGGVRTASGTTIKVSGRQAQGILLENPAAELQFRNGSVTSSGQLSDDGIRR
FLGTVTVKAGKLVADHATLANVGDTWDDDGIALYVAGEQAQASIADSTLQGAGGV
QIERGANVTVQRSAIVDGGLHIGALQSLQPEDLPPSRVVLRDTNVTAVPASGAPAAV
SVLGASELTLDGGHITGGRAAGVAAMQGAVVHLQRATIRRGDAPAGGAVPGGAVP
GGAVPGGFGPGGFGPVLDGWYGVDVSGSSVELAQSIVEAPELGAAIRVGRGARVTV
SGGSLSAPHGNVIETGGARRFAPQAAPLSITLQAGAHAQGKALLYRVLPEPVKLTLT
GGADAQGDIVATELPSIPGTSIGPLDVALASQARWTGATRAVDSLSIDNATWVMTDN
SNVGALRLASDGSVDFQQPAEAGRFKVLTVNTLAGSGLFRMNVFADLGLSDKLVV
MQDASGQHRLWVRNSGSEPASANTLLLVQTPLGSAATFTLANKDGKVDIGTYRYRL
AANGNGQWSLVGAKAPPO

FIGURE 22A
DNA CONSTRUCTS ENCODING PRION CHIMERAS

Construct designated Tpa.mprpf1gca (SEQ ID NO:171):

GAATTCGCCACCATGGATGCAATGAAGAGAGGGCTCTGCTGTGTGCTGCTGCTGT
GTGGAGCAGTCTTCGTTTCGCCCAGCGCTAGCAGGCCCATGATACATTTCGGCAA
TGACTGGGAGGACCGTTACTATCGTGAAAACATGTATCGTACAGCCTATATTGAC
CCCCAGGCTTCAGTCATAGGAGAGGTGACAATAGGTGCCAACGTTATGGTTTCCC
CCATGGCATCTATTAGGAGTGACGAAGGAATGCCAATTTTCGTAGGAGACAGAA
GCAATGTCCAGGATGGAGTTGTGCTTCATGCCCTTGAGACAATAAATGAAGAAG
GCGAACCTATAGAAGACAACATTGTTGAAGTTGATGGCAAGGAATACGCAGTCT
ATATAGGAAATAATGTTTCGCTTGCTCATCAATCCCAGGTTCATGGTCCGGCGGC
AGTAGGCGATGATACATTTATCGGTATGCAGGCTTTCGTTTTCAAGTCAAAGGTA
GGGAACAACTGTGTACTTGAGCCGAGATCGGCAGCAATCGGTGTAACCATTCCT
GACGGCCGGTATATCCCGGCAGGCATGGTTGTCACTTCGCAGGCCGAAGCCGAT
AAGCTGCCAGAGGTCACCGATGATTACGCATACAGCCATACTAACGAAGCCGTA
GTATATGTAAACGTCCACCTTGCCGAGGGTTATAAAGAAACTTCATAGGATCC

Prion chimera encoded by construct designated Tpa.mprpf1gca (SEQ ID NO:142):

MDAMKRGLCCVLLLCGAVFVSPSASRPMIHFGNDWEDRYYRENMYRTAYIDPQAS
VIGEVTIGANVMVSPMASIRSDEGMPIFVGDRSNVQDGVVLHALETINEEGEPIEDNI
VEVDGKEYAVYIGNNVSLAHQSQVHGPAAVGDDTFIGMQAFVFKSKVGNNCVLEP
RSAAIGVTIPDGRYIPAGMVVTSQAEADKLPEVTDDYAYSHTNEAVVYVNVHLAEG
YKETSO

Construct designated Tpa.mprpf2.gca (SEQ ID NO:172):

GAATTCGCCACCATGGATGCAATGAAGAGAGGGCTCTGCTGTGTGCTGCTGCTGT
GTGGAGCAGTCTTCGTTTCGCCCAGCGCTAGCAGGCCCATGATACATTTCGGCAA
TGACTGGGAGGACCGTTACTATCGTGAAAACATGTATCGTGCCTATATTGACCCC
CAGGCTTCAGTCATAGGAGAGGTGACAATAGGTGCCAACGTTATGGTTTCCCCCA
TGGCATCTATTAGGAGTGACGAAGGAATGCCAATTTTCGTAGGAGACAGAAGCA
ATGTCCAGGATGGAGTTGTGCTTCATGCCCTTGAGACAATAAATGAAGAAGGCG
AACCTATAGAAGACAACATTGTTGAAGTTGATGGCAAGGAATACGCAGTCTATA
TAGGAAATAATGTTTCGCTTGCTCATCAATCCCAGGTTCATGGTCCGGCGGCAGT
AGGCGATGATACATTTATCGGTATGCAGGCTTTCGTTTTCAAGTCAAAGGTAGGG
AACAACTGTGTACTTGAGCCGAGATCGGCAGCAATCGGTGTAACCATTCCTGACG
GCCGGTATATCCCGGCAGGCATGGTTGTCACTTCGCAGGCCGAAGCCGATAAGCT
GCCAGAGGTCACCGATGATTACGCATACAGCCATACTAACGAAGCCGTAGTATA
TGTAAACGTCCACCTTGCCGAGGGTTATAAAGAAACTTCATAGGATCC

Prion chimera encoded by construct designated Tpa.mprpf2.gca (SEQ ID NO:143):

MDAMKRGLCCVLLLCGAVFVSPSASRPMIHFGNDWEDRYYRENMYRAYIDPQASVI
GEVTIGANVMVSPMASIRSDEGMPIFVGDRSNVQDGVVLHALETINEEGEPIEDNIVE
VDGKEYAVYIGNNVSLAHQSQVHGPAAVGDDTFIGMQAFVFKSKVGNNCVLEPRS
AAIGVTIPDGRYIPAGMVVTSQAEADKLPEVTDDYAYSHTNEAVVYVNVHLAEGYK
ETSO

FIGURE 22B
DNA CONSTRUCTS ENCODING PRION CHIMERAS

Construct designated Tpa.mprpf3.gca (SEQ ID NO:173):

GAATTCGCCACCATGGATGCAATGAAGAGAGGGCTCTGCTGTGTGCTGCTGCTGT
GTGGAGCAGTCTTCGTTTCGCCCAGCGCTAGCAGGCCCATGATACATTTCGGCAA
TGACTGGGAGGACCGTTACTATCGTGAAAACATGTATCGTTATATTGACCCCCAG
GCTTCAGTCATAGGAGAGGTGACAATAGGTGCCAACGTTATGGTTTCCCCCATGG
CATCTATTAGGAGTGACGAAGGAATGCCAATTTTCGTAGGAGACAGAAGCAATG
TCCAGGATGGAGTTGTGCTTCATGCCCTTGAGACAATAAATGAAGAAGGCGAAC
CTATAGAAGACAACATTGTTGAAGTTGATGGCAAGGAATACGCAGTCTATATAG
GAAATAATGTTTCGCTTGCTCATCAATCCCAGGTTCATGGTCCGGCGGCAGTAGG
CGATGATACATTTATCGGTATGCAGGCTTTCGTTTTCAAGTCAAAGGTAGGGAAC
AACTGTGTACTTGAGCCGAGATCGGCAGCAATCGGTGTAACCATTCCTGACGGCC
GGTATATCCCGGCAGGCATGGTTGTCACTTCGCAGGCCGAAGCCGATAAGCTGCC
AGAGGTCACCGATGATTACGCATACAGCCATACTAACGAAGCCGTAGTATATGT
AAACGTCCACCTTGCCGAGGGTTATAAAGAAACTTCATAGGATCC

Prion chimera encoded by construct designated Tpa.mprpf3gca (SEQ ID NO:144):

MDAMKRGLCCVLLLCGAVFVSPSASRPMIHFGNDWEDRYYRENMYRYIDPQASVIG
EVTIGANVMVSPMASIRSDEGMPIFVGDRSNVQDGVVLHALETINEEGEPIEDNIVEV
DGKEYAVYIGNNVSLAHQSQVHGPAAVGDDTFIGMQAFVFKSKVGNNCVLEPRSA
AIGVTIPDGRYIPAGMVVTSQAEADKLPEVTDDYAYSHTNEAVVYVNVHLAEGYKE
TSO

Construct designated Tpa.mprpf4.gca (SEQ ID NO:174):

GAATTCGCCACCATGGATGCAATGAAGAGAGGGCTCTGCTGTGTGCTGCTGCTGT
GTGGAGCAGTCTTCGTTTCGCCCAGCGCTAGCAGGCCCATGATACATTTCGGCAA
TGACTGGGAGGACCGTTACTATCGTGAAAACATGTATCGTATTGACCCCCAGGCT
TCAGTCATAGGAGAGGTGACAATAGGTGCCAACGTTATGGTTTCCCCCATGGCAT
CTATTAGGAGTGACGAAGGAATGCCAATTTTCGTAGGAGACAGAAGCAATGTCC
AGGATGGAGTTGTGCTTCATGCCCTTGAGACAATAAATGAAGAAGGCGAACCTA
TAGAAGACAACATTGTTGAAGTTGATGGCAAGGAATACGCAGTCTATATAGGAA
ATAATGTTTCGCTTGCTCATCAATCCCAGGTTCATGGTCCGGCGGCAGTAGGCGA
TGATACATTTATCGGTATGCAGGCTTTCGTTTTCAAGTCAAAGGTAGGGAACAAC
TGTGTACTTGAGCCGAGATCGGCAGCAATCGGTGTAACCATTCCTGACGGCCGGT
ATATCCCGGCAGGCATGGTTGTCACTTCGCAGGCCGAAGCCGATAAGCTGCCAG
AGGTCACCGATGATTACGCATACAGCCATACTAACGAAGCCGTAGTATATGTAA
ACGTCCACCTTGCCGAGGGTTATAAAGAAACTTCATAGGATCC

Prion chimera encoded by construct designated Tpa.mprpf4gca (SEQ ID NO:145):

MDAMKRGLCCVLLLCGAVFVSPSASRPMIHFGNDWEDRYYRENMYRIDPQASVIGE
VTIGANVMVSPMASIRSDEGMPIFVGDRSNVQDGVVLHALETINEEGEPIEDNIVEVD
GKEYAVYIGNNVSLAHQSQVHGPAAVGDDTFIGMQAFVFKSKVGNNCVLEPRSAAI
GVTIPDGRYIPAGMVVTSQAEADKLPEVTDDYAYSHTNEAVVYVNVHLAEGYKETS
O

FIGURE 22C
DNA CONSTRUCTS ENCODING PRION CHIMERAS

Construct designated Tpa.mprpf5.gca (SEQ ID NO:175):

GAATTCGCCACCATGGATGCAATGAAGAGAGGGCTCTGCTGTGTGCTGCTGCTGT
GTGGAGCAGTCTTCGTTTCGCCCAGCGCTAGCAGGCCCATGATACATTTCGGCAA
TGACTGGGAGGACCGTTACTATCGTGAAAACATGTATCGTGACCCCCAGGCTTCA
GTCATAGGAGAGGTGACAATAGGTGCCAACGTTATGGTTTCCCCCATGGCATCTA
TTAGGAGTGACGAAGGAATGCCAATTTTCGTAGGAGACAGAAGCAATGTCCAGG
ATGGAGTTGTGCTTCATGCCCTTGAGACAATAAATGAAGAAGGCGAACCTATAG
AAGACAACATTGTTGAAGTTGATGGCAAGGAATACGCAGTCTATATAGGAAATA
ATGTTTCGCTTGCTCATCAATCCCAGGTTCATGGTCCGGCGGCAGTAGGCGATGA
TACATTTATCGGTATGCAGGCTTTCGTTTTCAAGTCAAAGGTAGGGAACAACTGT
GTACTTGAGCCGAGATCGGCAGCAATCGGTGTAACCATTCCTGACGGCCGGTATA
TCCCGGCAGGCATGGTTGTCACTTCGCAGGCCGAAGCCGATAAGCTGCCAGAGG
TCACCGATGATTACGCATACAGCCATACTAACGAAGCCGTAGTATATGTAAACGT
CCACCTTGCCGAGGGTTATAAAGAAACTTCATAGGATCC

Prion chimera encoded by construct designated Tpa.mprpf5.gca (SEQ ID NO:146)

MDAMKRGLCCVLLLCGAVFVSPSASRPMIHFGNDWEDRYYRENMYRDPQASVIGE
VTIGANVMVSPMASIRSDEGMPIFVGDRSNVQDGVVLHALETINEEGEPIEDNIVEVD
GKEYAVYIGNNVSLAHQSQVHGPAAVGDDTFIGMQAFVFKSKVGNNCVLEPRSAAI
GVTIPDGRYIPAGMVVTSQAEADKLPEVTDDYAYSHTNEAVVYVNVHLAEGYKETS
O

Construct designated Tpa.mprpf6.gca (SEQ ID NO:176):

GAATTCGCCACCATGGATGCAATGAAGAGAGGGCTCTGCTGTGTGCTGCTGCTGT
GTGGAGCAGTCTTCGTTTCGCCCAGCGCTAGCAGGCCCATGATACATTTCGGCAA
TGACTGGGAGGACCGTTACTATCGTGAAAACATGTATCGTCCCCAGGCTTCAGTC
ATAGGAGAGGTGACAATAGGTGCCAACGTTATGGTTTCCCCCATGGCATCTATTA
GGAGTGACGAAGGAATGCCAATTTTCGTAGGAGACAGAAGCAATGTCCAGGATG
GAGTTGTGCTTCATGCCCTTGAGACAATAAATGAAGAAGGCGAACCTATAGAAG
ACAACATTGTTGAAGTTGATGGCAAGGAATACGCAGTCTATATAGGAAATAATG
TTTCGCTTGCTCATCAATCCCAGGTTCATGGTCCGGCGGCAGTAGGCGATGATAC
ATTTATCGGTATGCAGGCTTTCGTTTTCAAGTCAAAGGTAGGGAACAACTGTGTA
CTTGAGCCGAGATCGGCAGCAATCGGTGTAACCATTCCTGACGGCCGGTATATCC
CGGCAGGCATGGTTGTCACTTCGCAGGCCGAAGCCGATAAGCTGCCAGAGGTCA
CCGATGATTACGCATACAGCCATACTAACGAAGCCGTAGTATATGTAAACGTCCA
CCTTGCCGAGGGTTATAAAGAAACTTCATAGGATCC

Prion chimera encoded by construct designated Tpa.mprpf6.gca (SEQ ID NO:147):

MDAMKRGLCCVLLLCGAVFVSPSASRPMIHFGNDWEDRYYRENMYRPQASVIGEV
TIGANVMVSPMASIRSDEGMPIFVGDRSNVQDGVVLHALETINEEGEPIEDNIVEVDG
KEYAVYIGNNVSLAHQSQVHGPAAVGDDTFIGMQAFVFKSKVGNNCVLEPRSAAIG
VTIPDGRYIPAGMVVTSQAEADKLPEVTDDYAYSHTNEAVVYVNVHLAEGYKETSO

FIGURE 23A
DNA CONSTRUCTS ENCODING PRION CHIMERAS

Construct designated Tpa.gca.mprpf1 (SEQ ID NO:177):
GAATTCGCCACCATGGATGCAATGAAGAGAGGGCTCTGCTGTGTGCTGCTGCTGT
GTGGAGCAGTCTTCGTTTCGCCCAGCGCTAGCATGTTTAATAAGCAGATCTTTAC
AATTCTTATCCTTTCTCTTTCACTTGCTCTTGCAGGGAGCGGGTGCATCTCCGAAG
GAGCAGAGGATAATGTCGCACAGGAAATAACCGTGGATGAGTTTTCGAATATCA
GAGAGAATCCGGTAACACCCTGGAATCCCGAACCATCAGCACCTGTGATTGATC
CCACAGCCTATATTGACCCCCAGGCTTCAGTCATAGGAGAGGTGACAATAGGTG
CCAACGTTATGGTTTCCCCCATGGCATCTATTAGGAGTGACGAAGGAATGCCAAT
TTTCGTAGGAGACAGAAGCAATGTCCAGGATGGAGTTGTGCTTCATGCCCTTGAG
ACAATAAATGAAGAAGGCGAACCTATAGAAGACAACATTGTTGAAGTTGATGGC
AAGGAATACGCAGTCTATATAGGAAATAATGTTTCGCTTGCTCATCAATCCCAGG
TTCATGGTCCGGCGGCAGTAGGCGATGATACATTTATCGGTATGCAGGCTTTCGT
TTTCAAGTCAAAGGTAGGGAACAACTGTGTACTTGAGCCGAGATCGGCAGCAAT
CGGTGTAACCATTCCTGACGGCCGGTATATCCCGGCAGGCATGGTTGTCACTAGG
CCCATGATACATTTCGGCAATGACTGGGAGGACCGTTACTATCGTGAAAACATGT
ATCGTTAGGATCCACTACGCGT

Prion chimera encoded by construct designated Tpa.gca.mprpf1 (SEQ ID NO:148):
MDAMKRGLCCVLLLCGAVFVSPSASMFNKQIFTILILSLSLALAGSGCISEGAEDN
VAQEITVDEFSNIRENPVTPWNPEPSAPVIDPTAYIDPQASVIGEVTIGANVMVSPMAS
IRSDEGMPIFVGDRSNVQDGVVLHALETINEEGEPIEDNIVEVDGKEYAVYIGNNVSL
AHQSQVHGPAAVGDDTFIGMQAFVFKSKVGNNCVLEPRSAAIGVTIPDGRYIPAGM
VVTRPMIHFGNDWEDRYYRENMYRO

Construct designated Tpa.gca.mprpf2 (SEQ ID NO:178):
GAATTCGCCACCATGGATGCAATGAAGAGAGGGCTCTGCTGTGTGCTGCTGCTGT
GTGGAGCAGTCTTCGTTTCGCCCAGCGCTAGCATGTTTAATAAGCAGATCTTTAC
AATTCTTATCCTTTCTCTTTCACTTGCTCTTGCAGGGAGCGGGTGCATCTCCGAAG
GAGCAGAGGATAATGTCGCACAGGAAATAACCGTGGATGAGTTTTCGAATATCA
GAGAGAATCCGGTAACACCCTGGAATCCCGAACCATCAGCACCTGTGATTGATC
CCACAGCCTATATTGACCCCCAGGCTTCAGTCATAGGAGAGGTGACAATAGGTG
CCAACGTTATGGTTTCCCCCATGGCATCTATTAGGAGTGACGAAGGAATGCCAAT
TTTCGTAGGAGACAGAAGCAATGTCCAGGATGGAGTTGTGCTTCATGCCCTTGAG
ACAATAAATGAAGAAGGCGAACCTATAGAAGACAACATTGTTGAAGTTGATGGC
AAGGAATACGCAGTCTATATAGGAAATAATGTTTCGCTTGCTCATCAATCCCAGG
TTCATGGTCCGGCGGCAGTAGGCGATGATACATTTATCGGTATGCAGGCTTTCGT
TTTCAAGTCAAAGGTAGGGAACAACTGTGTACTTGAGCCGAGATCGGCAGCAAT
CGGTGTAACCATTCCTGACGGCCGGTATATCCCGGCAGGCATGGTTGTCAGGCCC
ATGATACATTTCGGCAATGACTGGGAGGACCGTTACTATCGTGAAAACATGTATC
GTTAGGATCC

Prion chimera encoded by construct designated Tpa.gca.mprpf2 (SEQ ID NO:149):
MDAMKRGLCCVLLLCGAVFVSPSASMFNKQIFTILILSLSLALAGSGCISEGAEDN
VAQEITVDEFSNIRENPVTPWNPEPSAPVIDPTAYIDPQASVIGEVTIGANVMVSPMAS
IRSDEGMPIFVGDRSNVQDGVVLHALETINEEGEPIEDNIVEVDGKEYAVYIGNNVSL
AHQSQVHGPAAVGDDTFIGMQAFVFKSKVGNNCVLEPRSAAIGVTIPDGRYIPAGM
VVRPMIHFGNDWEDRYYRENMYRO

FIGURE 23B
DNA CONSTRUCTS ENCODING PRION CHIMERAS

Construct designated Tpa.gca.mprpf3 (SEQ ID NO:179):
GAATTCGCCACCATGGATGCAATGAAGAGAGGGCTCTGCTGTGTGCTGCTGCTGT
GTGGAGCAGTCTTCGTTTCGCCCAGCGCTAGCATGTTTAATAAGCAGATCTTTAC
AATTCTTATCCTTTCTCTTTCACTTGCTCTTGCAGGGAGCGGGTGCATCTCCGAAG
GAGCAGAGGATAATGTCGCACAGGAAATAACCGTGGATGAGTTTTCGAATATCA
GAGAGAATCCGGTAACACCCTGGAATCCCGAACCATCAGCACCTGTGATTGATC
CCACAGCCTATATTGACCCCCAGGCTTCAGTCATAGGAGAGGTGACAATAGGTG
CCAACGTTATGGTTTCCCCCATGGCATCTATTAGGAGTGACGAAGGAATGCCAAT
TTTCGTAGGAGACAGAAGCAATGTCCAGGATGGAGTTGTGCTTCATGCCCTTGAG
ACAATAAATGAAGAAGGCGAACCTATAGAAGACAACATTGTTGAAGTTGATGGC
AAGGAATACGCAGTCTATATAGGAAATAATGTTTCGCTTGCTCATCAATCCCAGG
TTCATGGTCCGGCGGCAGTAGGCGATGATACATTTATCGGTATGCAGGCTTTCGT
TTTCAAGTCAAAGGTAGGGAACAACTGTGTACTTGAGCCGAGATCGGCAGCAAT
CGGTGTAACCATTCCTGACGGCCGGTATATCCCGGCAGGCATGGTTAGGCCCATG
ATACATTTCGGCAATGACTGGGAGGACCGTTACTATCGTGAAAACATGTATCGTT
AGGATCC

Prion chimera encoded by construct designated Tpa.gca.mprpf3 (SEQ ID NO:150):
MDAMKRGLCCVLLLCGAVFVSPSASMFNKQIFTILILSLSLALAGSGCISEGAEDN
VAQEITVDEFSNIRENPVTPWNPEPSAPVIDPTAYIDPQASVIGEVTIGANVMVSPMAS
IRSDEGMPIFVGDRSNVQDGVVLHALETINEEGEPIEDNIVEVDGKEYAVYIGNNVSL
AHQSQVHGPAAVGDDTFIGMQAFVFKSKVGNNCVLEPRSAAIGVTIPDGRYIPAGM
VRPMIHFGNDWEDRYYRENMYRO

Construct designated Tpa.gca.mprpf4 (SEQ ID NO:180):
GAATTCGCCACCATGGATGCAATGAAGAGAGGGCTCTGCTGTGTGCTGCTGCTGT
GTGGAGCAGTCTTCGTTTCGCCCAGCGCTAGCATGTTTAATAAGCAGATCTTTAC
AATTCTTATCCTTTCTCTTTCACTTGCTCTTGCAGGGAGCGGGTGCATCTCCGAAG
GAGCAGAGGATAATGTCGCACAGGAAATAACCGTGGATGAGTTTTCGAATATCA
GAGAGAATCCGGTAACACCCTGGAATCCCGAACCATCAGCACCTGTGATTGATC
CCACAGCCTATATTGACCCCCAGGCTTCAGTCATAGGAGAGGTGACAATAGGTG
CCAACGTTATGGTTTCCCCCATGGCATCTATTAGGAGTGACGAAGGAATGCCAAT
TTTCGTAGGAGACAGAAGCAATGTCCAGGATGGAGTTGTGCTTCATGCCCTTGAG
ACAATAAATGAAGAAGGCGAACCTATAGAAGACAACATTGTTGAAGTTGATGGC
AAGGAATACGCAGTCTATATAGGAAATAATGTTTCGCTTGCTCATCAATCCCAGG
TTCATGGTCCGGCGGCAGTAGGCGATGATACATTTATCGGTATGCAGGCTTTCGT
TTTCAAGTCAAAGGTAGGGAACAACTGTGTACTTGAGCCGAGATCGGCAGCAAT
CGGTGTAACCATTCCTGACGGCCGGTATATCCCGGCAGGCATGAGGCCCATGATA
CATTTCGGCAATGACTGGGAGGACCGTTACTATCGTGAAAACATGTATCGTTAGG
ATCC

Prion chimera encoded by construct designated Tpa.gca.mprpf4 (SEQ ID NO:151):
MDAMKRGLCCVLLLCGAVFVSPSASMFNKQIFTILILSLSLALAGSGCISEGAEDN
VAQEITVDEFSNIRENPVTPWNPEPSAPVIDPTAYIDPQASVIGEVTIGANVMVSPMAS
IRSDEGMPIFVGDRSNVQDGVVLHALETINEEGEPIEDNIVEVDGKEYAVYIGNNVSL
AHQSQVHGPAAVGDDTFIGMQAFVFKSKVGNNCVLEPRSAAIGVTIPDGRYIPAGMR
PMIHFGNDWEDRYYRENMYRO

FIGURE 23C
DNA CONSTRUCTS ENCODING PRION CHIMERAS

Construct designated Tpa.gca.mprpf5 (SEQ ID NO:181):
GAATTCGCCACCATGGATGCAATGAAGAGAGGGCTCTGCTGTGTGCTGCTGCTGT
GTGGAGCAGTCTTCGTTTCGCCCAGCGCTAGCATGTTTAATAAGCAGATCTTTAC
AATTCTTATCCTTTCTCTTTCACTTGCTCTTGCAGGGAGCGGGTGCATCTCCGAAG
GAGCAGAGGATAATGTCGCACAGGAAATAACCGTGGATGAGTTTTCGAATATCA
GAGAGAATCCGGTAACACCCTGGAATCCCGAACCATCAGCACCTGTGATTGATC
CCACAGCCTATATTGACCCCCAGGCTTCAGTCATAGGAGAGGTGACAATAGGTG
CCAACGTTATGGTTTCCCCCATGGCATCTATTAGGAGTGACGAAGGAATGCCAAT
TTTCGTAGGAGACAGAAGCAATGTCCAGGATGGAGTTGTGCTTCATGCCCTTGAG
ACAATAAATGAAGAAGGCGAACCTATAGAAGACAACATTGTTGAAGTTGATGGC
AAGGAATACGCAGTCTATATAGGAAATAATGTTTCGCTTGCTCATCAATCCCAGG
TTCATGGTCCGGCGGCAGTAGGCGATGATACATTTATCGGTATGCAGGCTTTCGT
TTTCAAGTCAAAGGTAGGGAACAACTGTGTACTTGAGCCGAGATCGGCAGCAAT
CGGTGTAACCATTCCTGACGGCCGGTATATCCCGGCAGGCAGGCCCATGATACAT
TTCGGCAATGACTGGGAGGACCGTTACTATCGTGAAAACATGTATCGTTAGGATC
C

Prion chimera encoded by construct designated Tpa.gca.mprpf5 (SEQ ID NO:152):
MDAMKRGLCCVLLLCGAVFVSPSASMFNKQIFTILILSLSLALAGSGCISEGAEDN
VAQEITVDEFSNIRENPVTPWNPEPSAPVIDPTAYIDPQASVIGEVTIGANVMVSPMSI
RSDEGMPIFVGDRSNVQDGVVLHALETINEEGEPIEDNIVEVDGKEYAVYIGNNVSL
AHQSQVHGPAAVGDDTFIGMQAFVFKSKVGNNCVLEPRSAAIGVTIPDGRYIPAGRP
MIHFGNDWEDRYYRENMYRO

Construct designated Tpa.gca.mprpf6 (SEQ ID NO:182):
GAATTCGCCACCATGGATGCAATGAAGAGAGGGCTCTGCTGTGTGCTGCTGCTGT
GTGGAGCAGTCTTCGTTTCGCCCAGCGCTAGCATGTTTAATAAGCAGATCTTTAC
AATTCTTATCCTTTCTCTTTCACTTGCTCTTGCAGGGAGCGGGTGCATCTCCGAAG
GAGCAGAGGATAATGTCGCACAGGAAATAACCGTGGATGAGTTTTCGAATATCA
GAGAGAATCCGGTAACACCCTGGAATCCCGAACCATCAGCACCTGTGATTGATC
CCACAGCCTATATTGACCCCCAGGCTTCAGTCATAGGAGAGGTGACAATAGGTG
CCAACGTTATGGTTTCCCCCATGGCATCTATTAGGAGTGACGAAGGAATGCCAAT
TTTCGTAGGAGACAGAAGCAATGTCCAGGATGGAGTTGTGCTTCATGCCCTTGAG
ACAATAAATGAAGAAGGCGAACCTATAGAAGACAACATTGTTGAAGTTGATGGC
AAGGAATACGCAGTCTATATAGGAAATAATGTTTCGCTTGCTCATCAATCCCAGG
TTCATGGTCCGGCGGCAGTAGGCGATGATACATTTATCGGTATGCAGGCTTTCGT
TTTCAAGTCAAAGGTAGGGAACAACTGTGTACTTGAGCCGAGATCGGCAGCAAT
CGGTGTAACCATTCCTGACGGCCGGTATATCCCGGCAAGGCCCATGATACATTTC
GGCAATGACTGGGAGGACCGTTACTATCGTGAAAACATGTATCGTTAGGATCC

Prion chimera encoded by construct designated Tpa.gca.mprpf6 (SEQ ID NO:153):
MDAMKRGLCCVLLLCGAVFVSPSASMFNKQIFTILILSLSLALAGSGCISEGAEDN
VAQEITVDEFSNIRENPVTPWNPEPSAPVIDPTAYIDPQASVIGEVTIGANVMVSPMAS
IRSDEGMPIFVGDRSNVQDGVVLHALETINEEGEPIEDNIVEVDGKEYAVYIGNNVSL
AHQSQVHGPAAVGDDTFIGMQAFVFKSKVGNNCVLEPRSAAIGVTIPDGRYIPARPM
IHFGNDWEDRYYRENMYRO

FIGURE 24

TPA LEADER SEQUENCE:

MDAMKRGLCCVLLLCGAVFVSPSAS (SEQ ID NO:154)

GCA LEADER SEQUENCE:

MMFNK (SEQ ID NO:209)

FIGURE 25A

PRION CHIMERAS USING GCA CONTROL B NO LEADER AND HUMAN PrP (135-155)

GCABnoL/HuPrP135-155 Chimera No. 1: His Tag – GCA Control B no leader – Hu PrP (135 – 155):

SEQ ID NO:185

MHHHHHHGGQEITVDEFSNIRENPVTPWNPEPSAPVIDPTAYIDPQASVIGEVTIGAN
VMVSPMASIRSDEGMPIFVGDRSNVQDGVVLHALETINEEGEPIEDNIVEVDGKEYA
VYIGNNVSLAHQSQVHGPAAVGDDTFIGMQAFVFKSKVGNNCVLEPRSAAIGVTIPD
GRYIPAGMVVTRPIIHFGSDYEDRYYRENMHR

GCABnoL/HuPrP135-155 Chimera No. 2: His Tag – GCA Control B no leader – Hu PrP (135 – 155):

SEQ ID NO:186

MHHHHHHGGQEITVDEFSNIRENPVTPWNPEPSAPVIDPTAYIDPQASVIGEVTIGAN
VMVSPMASIRSDEGMPIFVGDRSNVQDGVVLHALETINEEGEPIEDNIVEVDGKEYA
VYIGNNVSLAHQSQVHGPAAVGDDTFIGMQAFVFKSKVGNNCVLEPRSAAIGVTIPD
GRYIPAGMVVRPIIHFGSDYEDRYYRENMHR

GCABnoL/HuPrP135-155 Chimera No. 3: His Tag – GCA Control B no leader – Hu PrP (135 – 155):

SEQ ID NO:187

MHHHHHHGGQEITVDEFSNIRENPVTPWNPEPSAPVIDPTAYIDPQASVIGEVT
IGANVMVSPMASIRSDEGMPIFVGDRSNVQDGVVLHALETINEEGEPIEDNIVEVDGK
EYAVYIGNNVSLAHQSQVHGPAAVGDDTFIGMQAFVFKSKVGNNCVLEPRSAAIGV
TIPDGRYIPAGMVRPIIHFGSDYEDRYYRENMHR

FIGURE 25B

PRION CHIMERAS USING GCA CONTROL B NO LEADER AND HUMAN PrP (135-155)

GCABnoL/HuPrP135-155 Chimera No. 4: His Tag – GCA Control B no leader – Hu PrP (135 – 155):

SEQ ID NO:188

MHHHHHHGGAQEITVDEFSNIRENPVTPWNPEPSAPVIDPTAYIDPQASVIGEVTIGA
NVMVSPMASIRSDEGMPIFVGDRSNVQDGVVLHALETINEEGEPIEDNIVEVDGKEY
AVYIGNNVSLAHQSQVHGPAAVGDDTFIGMQAFVFKSKVGNNCVLEPRSAAIGVTIP
DGRYIPAGMRPIIHFGSDYEDRYYRENMHR

GCABnoL/HuPrP135-155 Chimera No. 5: His Tag – GCA Control B no leader – Hu PrP (135 – 155):

SEQ ID NO:189

MHHHHHHGGQEITVDEFSNIRENPVTPWNPEPSAPVIDPTAYIDPQASVIGEVTIGAN
VMVSPMASIRSDEGMPIFVGDRSNVQDGVVLHALETINEEGEPIEDNIVEVDGKEYA
VYIGNNVSLAHQSQVHGPAAVGDDTFIGMQAFVFKSKVGNNCVLEPRSAAIGVTIPD
GRYIPAGRPIIHFGSDYEDRYYRENMHR

GCABnoL/HuPrP135-155 Chimera No. 6: His Tag – GCA Control B no leader – Hu PrP (135 – 155):

SEQ ID NO:190

MHHHHHHGGQEITVDEFSNIRENPVTPWNPEPSAPVIDPTAYIDPQASVIGEVTIGAN
VMVSPMASIRSDEGMPIFVGDRSNVQDGVVLHALETINEEGEPIEDNIVEVDGKEYA
VYIGNNVSLAHQSQVHGPAAVGDDTFIGMQAFVFKSKVGNNCVLEPRSAAIGVTIPD
GRYIPARPIIHFGSDYEDRYYRENMHR

FIGURE 26A

PRION CHIMERAS USING GCA CONTROL B NO LEADER AND MOUSE PrP (135-155)

GCABnoL/MoPrP135-155 Chimera No. 1: His Tag – GCA Control B no leader – Mo PrP (135 – 155):

SEQ ID NO:191

MHHHHHHGGQEITVDEFSNIRENPVTPWNPEPSAPVIDPTAYIDPQASVIGEVTIGAN
VMVSPMASIRSDEGMPIFVGDRSNVQDGVVLHALETINEEGEPIEDNIVEVDGKEYA
VYIGNNVSLAHQSQVHGPAAVGDDTFIGMQAFVFKSKVGNNCVLEPRSAAIGVTIPD
GRYIPAGMVVTMRPMIHFGNDWEDRYYRENMYR

GCABnoL/MoPrP135-155 Chimera No. 2: His Tag – GCA Control B no leader – Mo PrP (135 – 155):

SEQ ID NO:192

MHHHHHHGGQEITVDEFSNIRENPVTPWNPEPSAPVIDPTAYIDPQASVIGEVTIGAN
VMVSPMASIRSDEGMPIFVGDRSNVQDGVVLHALETINEEGEPIEDNIVEVDGKEYA
VYIGNNVSLAHQSQVHGPAAVGDDTFIGMQAFVFKSKVGNNCVLEPRSAAIGVTIPD
GRYIPAGMVVTRPMIHFGNDWEDRYYRENMYR

GCABnoL/MoPrP135-155 Chimera No. 3: His Tag – GCA Control B no leader – Mo PrP (135 – 155):

SEQ ID NO:193

MHHHHHHGGQEITVDEFSNIRENPVTPWNPEPSAPVIDPTAYIDPQASVIGEVTIGAN
VMVSPMASIRSDEGMPIFVGDRSNVQDGVVLHALETINEEGEPIEDNIVEVDGKEYA
VYIGNNVSLAHQSQVHGPAAVGDDTFIGMQAFVFKSKVGNNCVLEPRSAAIGVTIPD
GRYIPAGMVVRPMIHFGNDWEDRYYRENMYR

FIGURE 26B

PRION CHIMERAS USING GCA CONTROL B NO LEADER AND MOUSE PrP (135-155)

GCABnoL/MoPrP135-155 Chimera No. 4: His Tag – GCA Control B no leader – Mo PrP (135 – 155):

SEQ ID NO:194

MHHHHHHGGQEITVDEFSNIRENPVTPWNPEPSAPVIDPTAYIDPQASVIGEVTIGAN
VMVSPMASIRSDEGMPIFVGDRSNVQDGVVLHALETINEEGEPIEDNIVEVDGKEYA
VYIGNNVSLAHQSQVHGPAAVGDDTFIGMQAFVFKSKVGNNCVLEPRSAAIGVTIPD
GRYIPAGMVRPMIHFGNDWEDRYYRENMYR

GCABnoL/MoPrP135-155 Chimera No. 5: His Tag – GCA Control B no leader – Mo PrP (135 – 155):

SEQ ID NO:195

MHHHHHHGGQEITVDEFSNIRENPVTPWNPEPSAPVIDPTAYIDPQASVIGEVTIGAN
VMVSPMASIRSDEGMPIFVGDRSNVQDGVVLHALETINEEGEPIEDNIVEVDGKEYA
VYIGNNVSLAHQSQVHGPAAVGDDTFIGMQAFVFKSKVGNNCVLEPRSAAIGVTIPD
GRYIPAGMRPMIHFGNDWEDRYYRENMYR

GCABnoL/MoPrP135-155 Chimera No. 6: His Tag – GCA Control B no leader – Mo PrP (135 – 155):

SEQ ID NO:196

MHHHHHHGGQEITVDEFSNIRENPVTPWNPEPSAPVIDPTAYIDPQASVIGEVT
IGANVMVSPMASIRSDEGMPIFVGDRSNVQDGVVLHALETINEEGEPIEDNIVEVDGK
EYAVYIGNNVSLAHQSQVHGPAAVGDDTFIGMQAFVFKSKVGNNCVLEPRSAAIGV
TIPDGRYIPAGRPMIHFGNDWEDRYYRENMYR

FIGURE 27A

PRION CHIMERAS USING GCA CONTROL B NO LEADER AND HUMAN PrP (126-154)

GCABnoL/HuPrP126-154 Chimera No. 1: His Tag – GCA Control B no leader – Hu PrP (126 – 154):

SEQ ID NO:197

MHHHHHHGGQEITVDEFSNIRENPVTPWNPEPSAPVIDPTAYIDPQASVIGEVTIGAN
VMVSPMASIRSDEGMPIFVGDRSNVQDGVVLHALETINEEGEPIEDNIVEVDGKEYA
VYIGNNVSLAHQSQVHGPAAVGDDTFIGMQAFVFKSKVGNNCVLEPRSAAIGVTIPD
GRYIPAGMVV**TGYMLGSAMSRPIIHFGSDYEDRYYRENMH**

GCABnoL/HuPrP126-154 Chimera No. 2: His Tag – GCA Control B no leader – Hu PrP (126 – 154):

SEQ ID NO:198

MHHHHHHGGQEITVDEFSNIRENPVTPWNPEPSAPVIDPTAYIDPQASVIGEVTIGAN
VMVSPMASIRSDEGMPIFVGDRSNVQDGVVLHALETINEEGEPIEDNIVEVDGKEYA
VYIGNNVSLAHQSQVHGPAAVGDDTFIGMQAFVFKSKVGNNCVLEPRSAAIGVTIPD
GRYIPAGMVV**GYMLGSAMSRPIIHFGSDYEDRYYRENMH**

GCABnoL/HuPrP126-154 Chimera No. 3: His Tag – GCA Control B no leader – Hu PrP (126 – 154):

SEQ ID NO:199

MHHHHHHGGQEITVDEFSNIRENPVTPWNPEPSAPVIDPTAYIDPQASVIGEVTIGAN
VMVSPMASIRSDEGMPIFVGDRSNVQDGVVLHALETINEEGEPIEDNIVEVDGKEYA
VYIGNNVSLAHQSQVHGPAAVGDDTFIGMQAFVFKSKVGNNCVLEPRSAAIGVTIPD
GRYIPAGMV**GYMLGSAMSRPIIHFGSDYEDRYYRENMH**

FIGURE 27B

PRION CHIMERAS USING GCA CONTROL B NO LEADER AND HUMAN PrP (126-154)

GCABnoL/HuPrP126-154 Chimera No. 4: His Tag – GCA Control B no leader – Hu PrP (126 – 154):

SEQ ID NO:200

MHHHHHHGGQEITVDEFSNIRENPVTPWNPEPSAPVIDPTAYIDPQASVIGEVTIGAN
VMVSPMASIRSDEGMPIFVGDRSNVQDGVVLHALETINEEGEPIEDNIVEVDGKEYA
VYIGNNVSLAHQSQVHGPAAVGDDTFIGMQAFVFKSKVGNNCVLEPRSAAIGVTIPD
GRYIPAGM**GYMLGSAMSRPIIHFGSDYEDRYYRENMH**

GCABnoL/HuPrP126-154 Chimera No. 5: His Tag – GCA Control B no leader – Hu PrP (126 – 154):

SEQ ID NO:201

MHHHHHHGGQEITVDEFSNIRENPVTPWNPEPSAPVIDPTAYIDPQASVIGEVTIGAN
VMVSPMASIRSDEGMPIFVGDRSNVQDGVVLHALETINEEGEPIEDNIVEVDGKEYA
VYIGNNVSLAHQSQVHGPAAVGDDTFIGMQAFVFKSKVGNNCVLEPRSAAIGVTIPD
GRYIPAG**GYMLGSAMSRPIIHFGSDYEDRYYRENMH**

GCABnoL/HuPrP126-154 Chimera No. 6: His Tag – GCA Control B no leader – Hu PrP (126 – 154):

SEQ ID NO:202

MHHHHHHGGQEITVDEFSNIRENPVTPWNPEPSAPVIDPTAYIDPQASVIGEVTIGAN
VMVSPMASIRSDEGMPIFVGDRSNVQDGVVLHALETINEEGEPIEDNIVEVDGKEYA
VYIGNNVSLAHQSQVHGPAAVGDDTFIGMQAFVFKSKVGNNCVLEPRSAAIGVTIPD
GRYIPA**GYMLGSAMSRPIIHFGSDYEDRYYRENMH**

FIGURE 28A

PRION CHIMERAS USING GCA CONTROL B NO LEADER AND MOUSE PrP (126-154)

GCABnoL/MoPrP126-154 Chimera No. 1: His Tag – GCA Control B no leader – Mo PrP (126 – 154):

SEQ ID NO:203

MHHHHHHGGQEITVDEFSNIRENPVTPWNPEPSAPVIDPTAYIDPQASVIGEVTIGAN
VMVSPMASIRSDEGMPIFVGDRSNVQDGVVLHALETINEEGEPIEDNIVEVDGKEYA
VYIGNNVSLAHQSQVHGPAAVGDDTFIGMQAFVFKSKVGNNCVLEPRSAAIGVTIPD
GRYIPAGMVVTM**GYMLGSAMSRPMIHFGNDWEDRYYRENMY**

GCABnoL/MoPrP126-154 Chimera No. 2: His Tag – GCA Control B no leader – Mo PrP (126 – 154):

SEQ ID NO:204

MHHHHHHGGQEITVDEFSNIRENPVTPWNPEPSAPVIDPTAYIDPQASVIGEVTIGAN
VMVSPMASIRSDEGMPIFVGDRSNVQDGVVLHALETINEEGEPIEDNIVEVDGKEYA
VYIGNNVSLAHQSQVHGPAAVGDDTFIGMQAFVFKSKVGNNCVLEPRSAAIGVTIPD
GRYIPAGMVVT**GYMLGSAMSRPMIHFGNDWEDRYYRENMY**

GCABnoL/MoPrP126-154 Chimera No. 3: His Tag – GCA Control B no leader – Mo PrP (126 – 154):

SEQ ID NO:205

MHHHHHHGGQEITVDEFSNIRENPVTPWNPEPSAPVIDPTAYIDPQASVIGEVTIGAN
VMVSPMASIRSDEGMPIFVGDRSNVQDGVVLHALETINEEGEPIEDNIVEVDGKEYA
VYIGNNVSLAHQSQVHGPAAVGDDTFIGMQAFVFKSKVGNNCVLEPRSAAIGVTIPD
GRYIPAGMVV**GYMLGSAMSRPMIHFGNDWEDRYYRENMY**

FIGURE 28B

PRION CHIMERAS USING GCA CONTROL B NO LEADER AND MOUSE PrP (126-154)

GCABnoL/MoPrP126-154 Chimera No. 4: His Tag – GCA Control B no leader – Mo PrP (126 – 154):

SEQ ID NO:206

MHHHHHHGGQEITVDEFSNIRENPVTPWNPEPSAPVIDPTAYIDPQASVIGEVTIGAN
VMVSPMASIRSDEGMPIFVGDRSNVQDGVVLHALETINEEGEPIEDNIVEVDGKEYA
VYIGNNVSLAHQSQVHGPAAVGDDTFIGMQAFVFKSKVGNNCVLEPRSAAIGVTIPD
GRYIPAGMV**GYMLGSAMSRPMIHFGNDWEDRYYRENMY**

GCABnoL/MoPrP126-154 Chimera No. 5: His Tag – GCA Control B no leader – Mo PrP (126 – 154):

SEQ ID NO:207

MHHHHHHGGQEITVDEFSNIRENPVTPWNPEPSAPVIDPTAYIDPQASVIGEVTIGAN
VMVSPMASIRSDEGMPIFVGDRSNVQDGVVLHALETINEEGEPIEDNIVEVDGKEYA
VYIGNNVSLAHQSQVHGPAAVGDDTFIGMQAFVFKSKVGNNCVLEPRSAAIGVTIPD
GRYIPAGM**GYMLGSAMSRPMIHFGNDWEDRYYRENMY**

GCABnoL/MoPrP126-154 Chimera No. 6: His Tag – GCA Control B no leader – Mo PrP (126 – 154):

SEQ ID NO:208

MHHHHHHGGQEITVDEFSNIRENPVTPWNPEPSAPVIDPTAYIDPQASVIGEVTIGAN
VMVSPMASIRSDEGMPIFVGDRSNVQDGVVLHALETINEEGEPIEDNIVEVDGKEYA
VYIGNNVSLAHQSQVHGPAAVGDDTFIGMQAFVFKSKVGNNCVLEPRSAAIGVTIPD
GRYIPAG**GYMLGSAMSRPMIHFGNDWEDRYYRENMY**

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US03/31057

A. CLASSIFICATION OF SUBJECT MATTER

IPC(7) : A61K 39/395; G01N 21/00, 33/53
US CL : 424/130.1, 141.1, 152.1; 422/61; 435/7.1

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
U.S. : 424/130.1, 141.1, 152.1; 422/61; 435/7.1

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
USPATFUL, WPIDS, MEDLINE

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	US 6,620,629 B1 (PRUSINER, S. B., et al.) 16 September 2003 (16.09.2003), see entire document, particularly columns 21-22.	1-58
Y	US 6,602,672 B1 (PRUSINER, S. B., et al.) 05 August 2003 (05.08.2003), see entire document, particularly columns 29-32.	1-58
Y	US 2002/0150571 A1 (PRUSINER, S. B., et al.) 17 October 2002 (17.10.2002), see entire document, particularly page 23.	1-58

☐ Further documents are listed in the continuation of Box C. ☐ See patent family annex.

* Special categories of cited documents:

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier application or patent published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search
10 September 2004 (10.09.2004)

Date of mailing of the international search report
30 SEP 2004

Name and mailing address of the ISA/US
Mail Stop PCT, Attn: ISA/US
Commissioner for Patents
P.O. Box 1450
Alexandria, Virginia 22313-1450
Facsimile No. (703) 305-3230

Authorized officer
JS Parkin
Telephone No. (703) 308-1141

Form PCT/ISA/210 (second sheet) (July 1998)